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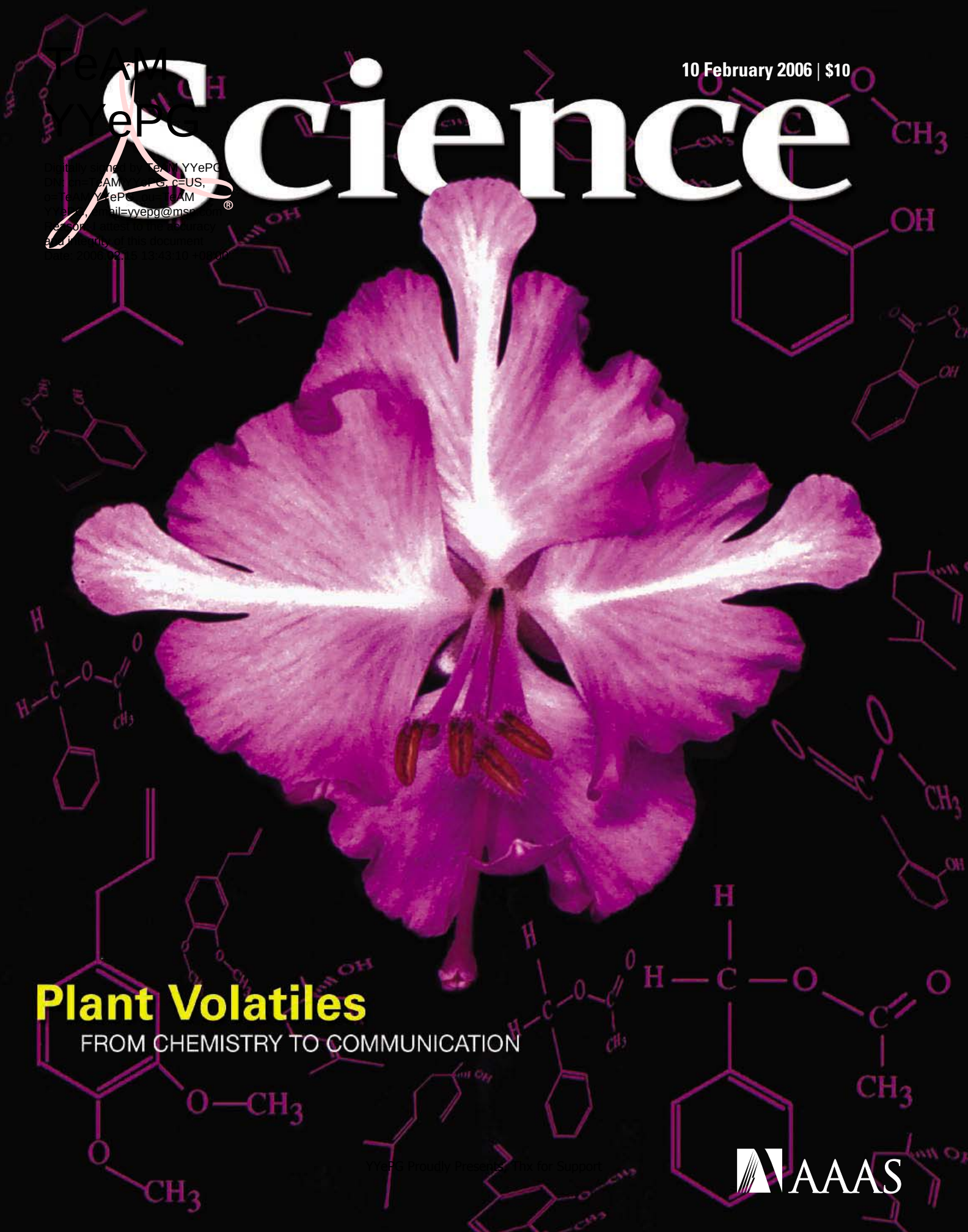

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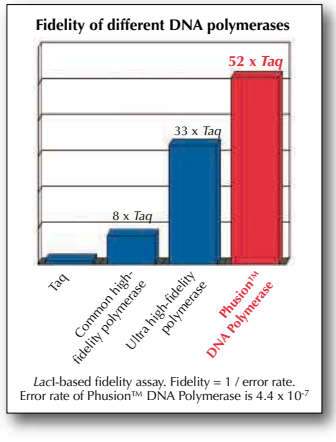

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Volume 311, Issue 5762

COVER

Clarkia breweri, a California annual, is a small plant that is pollinated by hawkmoths. Its intensely scented flowers synthesize more than 10 different volatile compounds. As discussed in the special section in this issue, this plant is one of several used for the study of the volatile chemicals produced by plants for communication and defense. See page 803.

Image: David Bay and Eran Pichersky

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For related online content in STKE, SAGE KE, and ScienceCareers, see page 735 or go to www.sciencemag.org/sciext/plantvolatiles/


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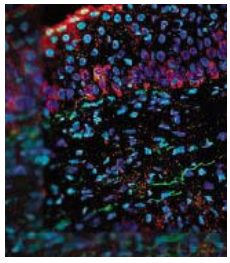
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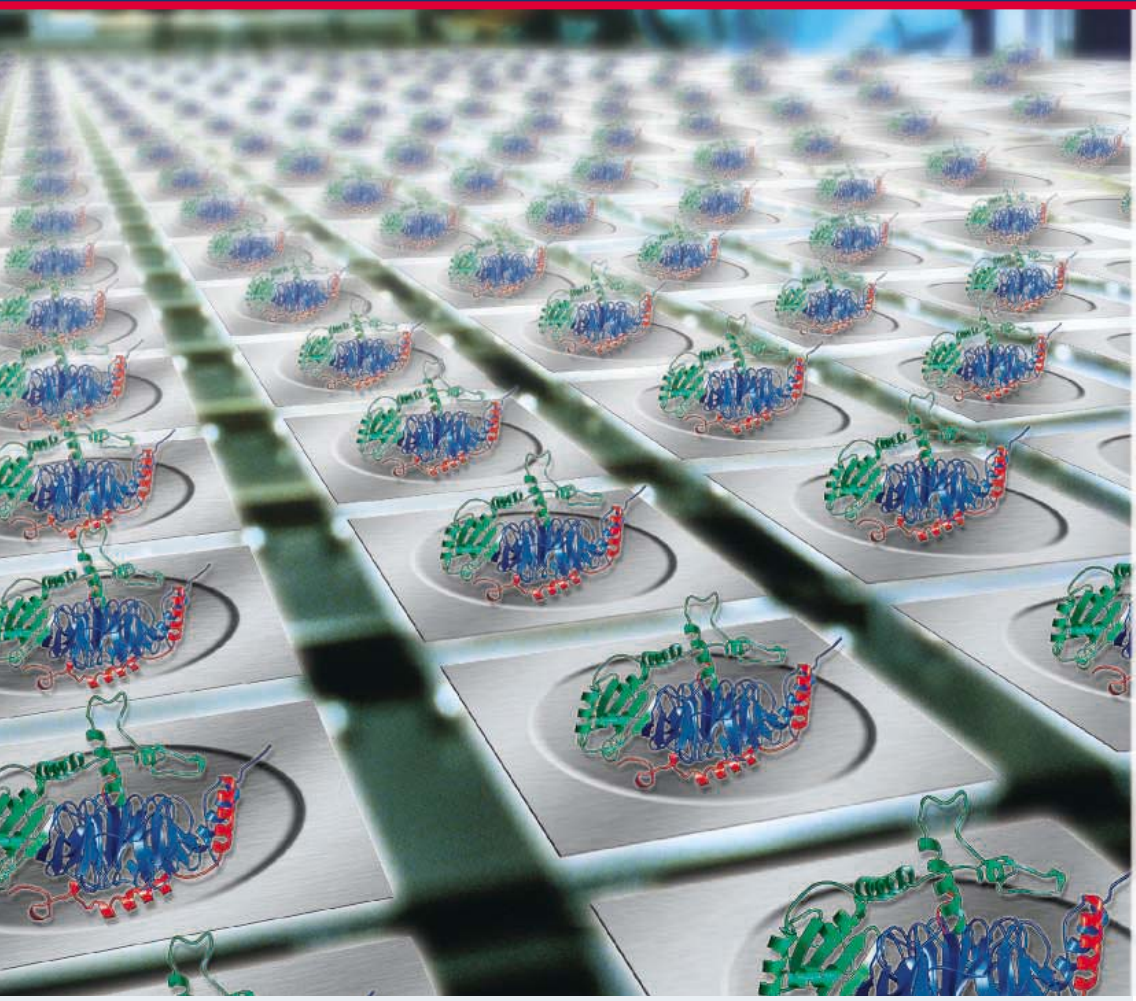
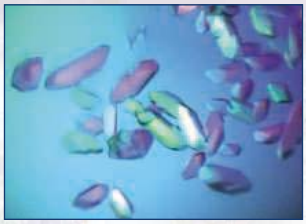
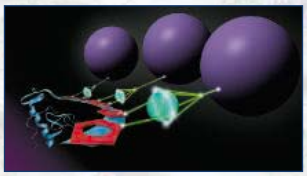
Standardized solutions for proteins





E. coli gyrase A C-terminal domain crystals. Courtesy of Alex Ruthenburg from Prof. Verdine's laboratory, Harvard University, Boston, USA.



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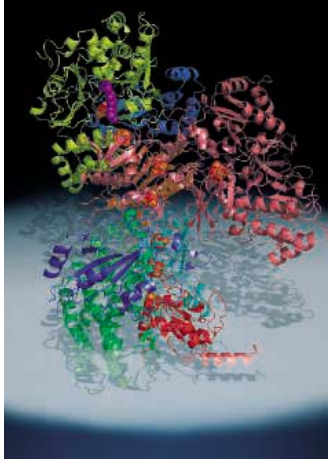

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CELL BIOLOGY

Progressive Disruption of Cellular Protein Folding in Models of Polyglutamine Diseases

T. Gidalevitz, A. Ben-Zvi, K. H. Ho, H. R. Brignull, R. I. Morimoto

In experiments in nematodes that may simulate some neurodegenerative diseases, abnormal, glutamine-rich proteins disrupt the cell's normal disposal of misfolded proteins.

10.1126/science.1124514

CHEMISTRY

Reactive and Nonreactive Scattering of H_2 from a Metal Surface Is Electronically Adiabatic

P. Nieto et al.

The interaction of H_2 with a platinum surface can be accurately modeled by treating electronic and nuclear motion as separate, confirming a basic approximation in chemical modeling.

10.1126/science.1123057

STRUCTURAL BIOLOGY

Structure of the Hydrophilic Domain of Respiratory Complex I from *Thermus Thermophilus*

L. A. Sazanov and P. Hinchliffe

The x-ray crystal structure of the peripheral part of the largest bacterial respiratory electron-transport complex shows the folds, contacts, and positions of the redox cofactors.

10.1126/science.1123809

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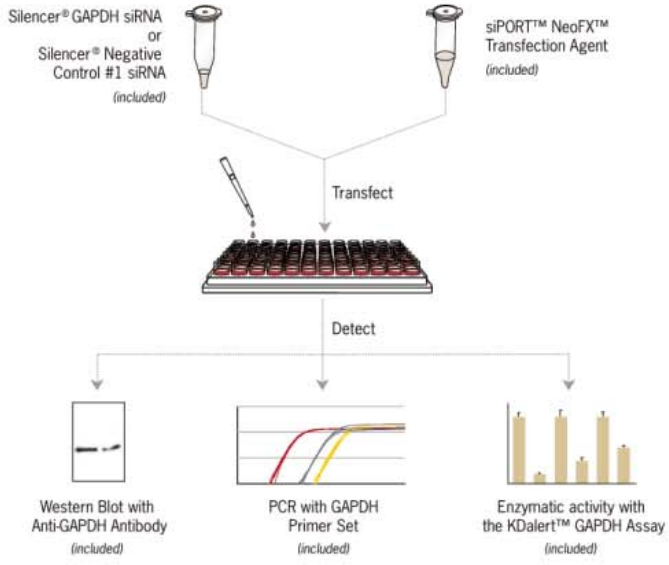

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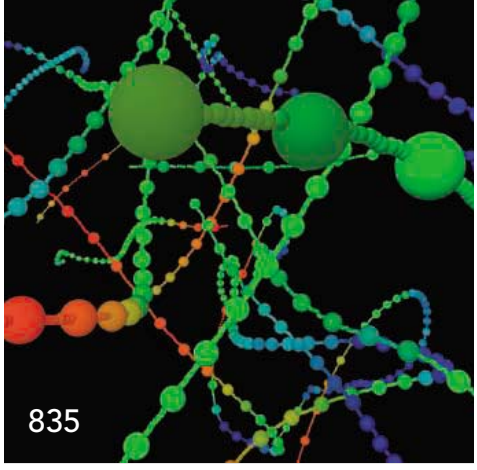

REPORTS

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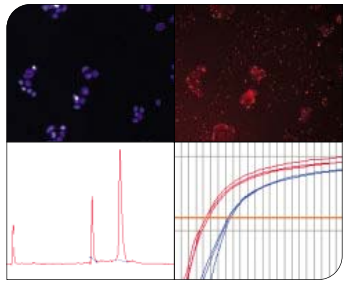

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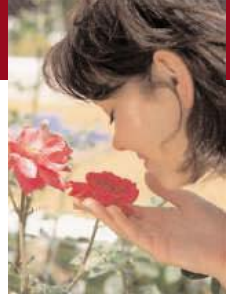

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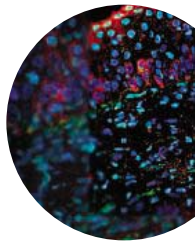

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Why does perception of volatile stimuli decline with age?

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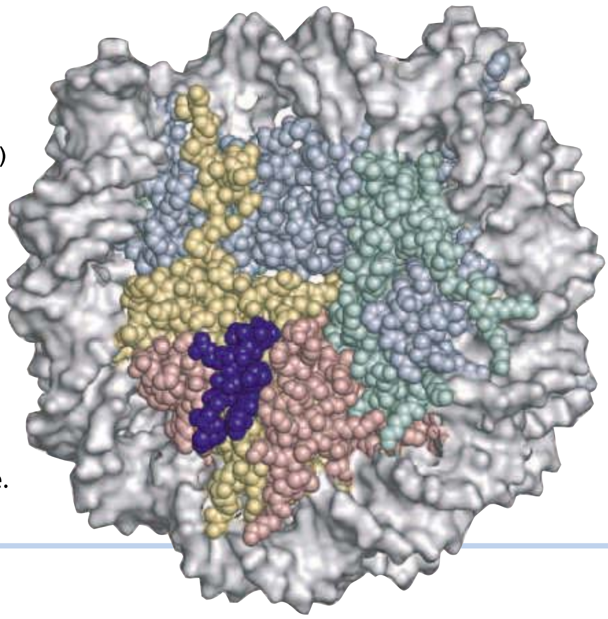
EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Hitching a Ride on the Chromosome

The Kaposi's sarcoma–associated herpesvirus (KSV) does not integrate into its host but is maintained as a stable episome. In order to be distributed to daughter cells, the virus associates with human chromosomes. **Barbera *et al.*** (p. 856) show that the viral latency-associated nuclear antigen (LANA) binds directly to specific chromosome components, the core histones H2A and H2B. LANA could not bind in systems that lack these two histones. The crystal structure of the complex revealed that a hairpin formed when LANA interacts with a particular acidic region formed by H2A and H2B within the nucleosome.

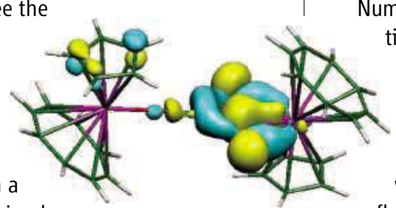


Cosmic Magnetism

Primordial magnetic fields arose in the hot young universe as a by-product of the gravitational collapse of cosmic structures. **Ichiki *et al.*** (p. 827, published online 5 January; see the Perspective by **Durrer**) show that primordial magnetic fields are strong enough to explain the fields seen in galaxy clusters and galaxies today. For a range of cosmic scales, they calculate how seed magnetic fields are produced by currents caused by the differing motions of charged protons and electrons as photons scattered off them during cosmic epochs before the first atoms formed.

Assembling a CO Triangle

The Fischer-Tropsch process uses catalysts and high temperature and pressure conditions to synthesize hydrocarbons from CO and H_2. However, efforts to link CO units more selectively under milder conditions have been largely unsuccessful, in part because of the high strength of the CO triple bond. **Summerscales *et al.*** (p. 829; see the Perspective by **Wayland and Fu**) have used a uranium complex to assemble three CO units in a triangular ring joined through the carbons and suspended between two U centers, each of which donates an electron to produce a $(CO)_3^{2-}$ dianion. Structural data and density functional theory suggest that uranium f orbitals are especially suited to stabilizing the structure.



CREDITS (TOP TO BOTTOM): BARBERA *ET AL.*; SUMMERSCALES *ET AL.*

Swiveling in a Net

Liquid water is held together by a net of intermolecular hydrogen (H) bonds that constantly break and reassemble. Rotation of water molecules would seem to require small diffusive steps as donated H-bonds are gradually transferred between acceptors. Numerical simulations by **Laage and Hynes** (p. 832, published online 26 January 2006) support a more delocalized mechanism in which rotation is controlled by coordination changes at the H-bond accepting partners in the solvation shell. Thus, rotation is generally restricted, but when bulk coordination is simultaneously added to the current acceptor and removed from a nearby potential acceptor, the donor molecule rapidly swivels from one to the other.

Shooting Methane Blanks

Numerous rapid increases in the concentration of atmospheric methane occurred during the last glacial period and deglaciation, associated with abrupt climate warming events. The "clathrate gun" hypothesis argues that the source was methane clathrates below the sea floor that were rapidly destabilized by ocean warming. **Sowers** (p. 838) tested that hypothesis with measurements of the isotopic composition of hydrogen in methane trapped in bubbles of the GISP2 Greenland ice core for several episodes of rapid warming during the last glacial period and the last deglaciation. He finds no evidence that methane clathrates, which have a unique hydrogen isotopic signature, contributed significantly to the methane concentration peaks.

In a Wider Warm Spell

A number of unusually warm or cold intervals can be seen in most proxy records of temperature of the last millennium, so how can we assess the relative magnitude of the current warm period? **Osborn and Briffa** (p. 841) compared the geographic extent of late 20th-century warming in the Northern Hemisphere to the distribution of both warm and cold intervals for the last 1200 years by adopting specific thresholds to define warm and cold periods in order to avoid questions about of the absolute magnitude of warm and cold events, and they considered only a subset of the data chosen specifically for its value as a temperature proxy. They find that the continuing warmth of the late 20th century is the most widespread and longest temperature anomaly of any kind since the 9th century A.D.

Modulating the Scaffold

Signaling complexes are often preassembled into complexes. So-called scaffold proteins help to maintain these complexes and can contribute to specificity in various signaling systems. **Bhattacharyya *et al.*** (p. 822, published online 19 January; see the Perspective by **Breitkreutz and Tyers**) show that the role of such scaffolds can go

Continued on page 739

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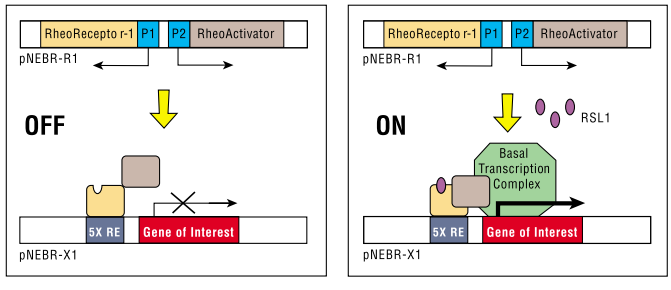

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Continued from page 737

beyond support and spatial localization. In yeast, mating pheromone causes activation of a series of kinases that all interact with the scaffold protein Ste5, and signal transduction through this pathway activates the mitogen-activated protein kinase Fus3. When Fus3 binds to Ste5, this interaction causes an allosteric partial activation of Fus3's kinase activity. Fus3 then appears to provide negative feedback in the system by phosphorylating the Ste5 scaffold.

Basic Body Design

Why have certain features of animal body plans, such as bilateral symmetry, been conserved since the early Cambrian period, whereas at the species level, there has been a continuous accumulation of changes? **Davidson and Erwin** (p. 796) propose that the genetic regulatory networks associated with development contain three components that differ in their evolutionary conservation. Evolutionarily inflexible subcircuits ("kernels") perform essential upstream functions in building given body parts, while other small subcircuits ("plug-ins") have been repeatedly co-opted to diverse developmental purposes, leaving highly flexible, individual cis-regulatory linkages to regulate detailed phenotypic variation.

CREDIT: DAVIDSON AND ERWIN

Self-Promoting Signals

Release of proapoptotic factors from the mitochondria leads to cell death, and signaling events appear to occur "upstream" or "downstream" of the mitochondria. This neat organization is challenged by **Lakhani *et al.*** (p. 847; see the Perspective by **Adrain and Martin**) in an analysis of knockout mice lacking caspase 3 and caspase 7, both thought to be "downstream." Caspases 3 and 7 are activated when clipped by other caspases after they have been stimulated by molecules released from the mitochondria. In the knockout animals, not only was the "downstream" event, apoptosis, inhibited, but "upstream" events, such as loss of the integrity of the mitochondrial membrane and release apoptotic factors, were also delayed. These unanticipated results may indicate that caspase 3 and caspase 7 act to promote mitochondrial signals that lead to their own activation and raise a "chicken or egg" conundrum regarding the initiation of the mitochondrial death signals.

Role for Translation in Maintaining Totipotency

Germ cells are totipotent—they can give rise to all different cell types. **Ciosk *et al.*** (p. 851) now show that the translational regulators MEX-3 and GLD-1 maintain totipotency in the germ line of the nematode *Caenorhabditis elegans*. When these two factors were eliminated, ectopic cells were found in the gonad due to the differentiation of germ cells into somatic cell types such as muscle, neurons, and intestinal cells. This transdifferentiation was associated with a loss of germ cell features such as P granules and germ cell proteins. These "worm teratomas" may be useful as a genetically tractable model system for understanding teratoma biology.

Word on the Street

To understand what forces control the emergence of extraordinarily successful songs, movies, or plays, **Salganik *et al.*** (p. 854; see the Perspective by **Hedström**) have assessed the influence of social information, that is, information about what other people are watching and listening to, on market performance. By querying students online about their assessments of a defined set of songs, the authors show that access to social information increases the tendency for certain songs to do well, and that the quality of the song is only partly reflected in its market performance.

Depressed Mouse Needs Long-Term Treatment

What are the neurobiological mechanisms through which psychosocial experience may alter the activity of the mesolimbic dopamine system? **Berton *et al.*** (p. 864; see the news story by **Holden**) demonstrate that long-lasting behavioral and molecular changes develop in mice after suffering a series of aggressive encounters. The persistent social aversion seen in these mice can be completely normalized by chronic (but not acute) treatment with clinically effective antidepressants. The growth factor brain-derived neurotrophic factor (BDNF) is required within dopaminergic reward regions for these behavioral alterations to unfold.

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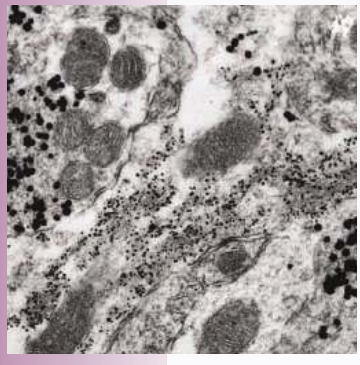

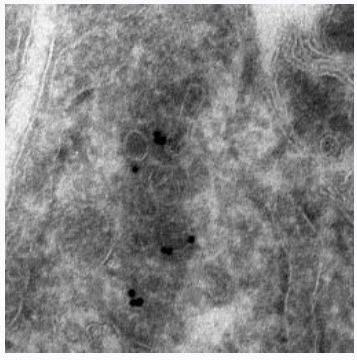

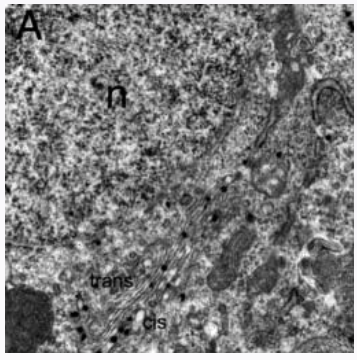

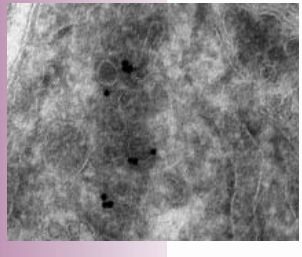

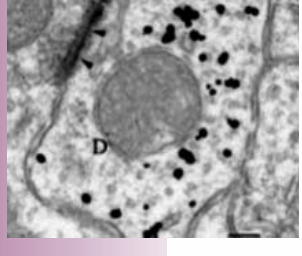

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Alan I. Leshner is chief executive officer of AAAS and executive publisher of *Science.*

Gilbert S. Omenn is president of AAAS and professor of medicine at the University of Michigan.

Seizing the Opportunities

THIS YEAR'S ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (AAAS) celebrates "Grand Challenges, Great Opportunities." The program was designed to challenge scientists, engineers, teachers, and citizens to approach major scientific and societal problems in ways that create opportunities to apply the best in science and technology for broad public benefit. The meeting showcases a diverse array of important scientific findings and provocative questions and emphasizes the enormous potential of modern science to advance all aspects of life around the world.

That potential has been heralded in recent public statements by both science and policy leaders and in formal reports that have been widely quoted by the media. Those reports, however, not only emphasize the great opportunities. They also point out the very real danger that those challenges will go unmet and those opportunities will be lost unless the nations of the world focus seriously and urgently on improving the infrastructure for science, engineering, and innovation.

The October 2005 report *Rising Above the Gathering Storm*, from the U.S. National Academy of Sciences, National Academy of Engineering, and Institute of Medicine, identifies two key challenges facing the United States that are tightly linked to science and engineering capabilities: creating and sustaining high-quality jobs for Americans and meeting the nation's need for clean, affordable, reliable energy. The report argues that America must strengthen its commitment to long-term basic research; develop, recruit, and retain top students, scientists, and engineers from both the United States and abroad; dramatically improve K–12 mathematics and science education for all students; and ensure that the United States remains the premier place in the world for innovation. The report lays out a series of actions to meet those goals, which AAAS strongly supports.

Similarly, the National Summit on Competitiveness, held at the U.S. Department of Commerce in December 2005, began its report with the message: "If trends in U.S. research and education continue, our nation will squander its economic leadership, and the results will be a lower standard of living for the American people." The summit urged specific actions to revitalize fundamental research, expand the U.S. innovation talent pool, and enable the United States to lead the world in the development and deployment of advanced technologies. In its 125th anniversary issue last year, *Science* sought to stimulate scientific risk-taking and creativity by highlighting 125 compelling questions about "What We Don't Know."

Many policy-makers recognize that the nations of the world must ensure that we collectively seize the opportunities embedded in modern science and engineering research and technology. In the U.S. Congress, there has been a flurry of bipartisan bills to authorize programs that could achieve the science and engineering infrastructure development goals laid out in these reports. Some have focused on individual scientific agencies, including the National Science Foundation, the U.S. Department of Energy, and the National Institutes of Health, whereas others have been broader in scope. The U.S. president's 2006 State of the Union Address last week outlined an American Competitiveness Initiative that could substantially increase support for fundamental research in the physical sciences and for science education, and enact a permanent tax credit for industrial R&D.

Our nation faces a distressing reality test: Although some U.S. policy-makers are working to authorize badly needed new programs and strengthen effective existing ones, the most recent U.S. budgets actually appropriated for science and engineering research and innovation (other than those directly related to homeland security or the military) have been either flat or decreasing in real dollars. Essentially everyone recognizes the importance of protection against security threats both at home and abroad. However, we must remind ourselves that our security also depends on the health and economic competitiveness of our people. We must find the political will to make the investments that will invigorate fundamental and translational research, strengthen science education, and create a more supportive climate for innovation, thereby meeting the national and global challenges to our economic security and exploiting the great opportunities in science and engineering that we proudly identify.

– Alan I. Leshner and Gilbert S. Omenn

10.1126/science.1125410

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HIGHLIGHTS OF THE RECENT LITERATURE

ECOLOGY/EVOLUTION

A Need for Specialists and Generalists

Raphanus raphanistrum **(above) and** ***Medicago sativa*****.**

No one disputes the agricultural importance of pollination, but what might happen if, under the current mass extinction, pollinator diversity were compromised? Fontaine *et al.* have measured the effect of pollinator diversity on plant yields in a 2-year experiment in caged plots at a site outside Paris. They created unmixed and mixed communities of plants with open or tubular flowers and pollinator insects with long (bumblebees) or short (syrphid hoverflies) proboscises, and they counted the number and species of fruits, seeds, and seedlings produced. As expected, the type of pollinator did have a significant effect: Bumblebees stimulated more fruit production overall, and the tubular flowers were unable to form fruits well if only syrphids were present. But there were unexpected effects: Although able to trigger fruit production, the bumblebees gave rise to fewer seeds per fruit for the open-flowered plants (possibly because they kept revisiting the same flowers, which is called geitonogamy), and when both types of pollinators were present, the overall recruitment of seedlings was enhanced, especially in the most complex of the communities. It appears that in mixed plots, the bumblebees show a preference for the tubular flowers and hence reduce their frequency of visits to the open flowers, which leaves the open flowers to the more efficient attentions of the syrphids. — CA

PLoS Biol. **4**, e1 (2006).

BIOCHEMISTRY

Filled with Lipids

The F- and V-type proton-pumping ATPases exhibit a common mechanical design in which the transmembrane passage of protons turns a membrane-embedded rotor that drives the nucleotide-binding components of the cytoplasmic turret through a cycle of conformational changes. This motor can run in forward or reverse directions, hydrolyzing ATP as it pumps protons uphill or making ATP as protons flow downhill. The precise structure of the entire membrane assembly has not yet been determined, but recent findings have offered views of the homo-oligomeric ring, which contains from 10 to 14 identical c subunits, depending on species. Using a photogenerated carbene, Oberfeld *et al.* fill in one of the gaps by demonstrating that in the *Escherichia coli* F-ATPase, the c subunits can be cross-linked to phospholipids at the inner surface of the ring, which is large enough (about 15 to 20 Å in diameter) to accommodate about 10 lipid molecules in the outer leaflet and 2 or 3 in the inner leaflet. — GJC

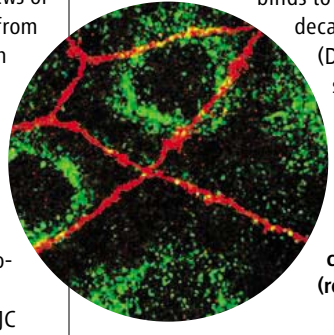
Biochemistry 10.1021/bi052304+ (2006).

VIROLOGY

Breaking and Entering

In order to establish a productive infection, group B coxsackie viruses (CVBs) (human pathogens that cause meningitis) need to cross an epithelial cell layer during transmission by fecal-oral or respiratory routes. Epithelial cells form a barrier to the passage of molecules and viruses by virtue of tight junctions that effectively seal off one side from the other. Protein components of the tight junction include the coxsackie and adenovirus receptor (CAR), whose virus-binding site is exposed only toward the basolateral surface; viruses approaching from the apical surface (the more likely arrival route) will not be able to access CAR.

Coyne and Bergelson describe how CVBs circumvent this problem of access. Invading virus binds to a protein known as decay-accelerating factor (DAF) on the apical surface of the epithelial cell layer. Binding to DAF triggers the intracellular activation of the Abl kinase, which promotes the rearrangement of the actin cytoskeleton via its effects on the small GTP-binding protein Rac. The actin rearrangements allow the virus to move to the tight junctions, where it can associate with CAR, which leads to virus entry and the eventual release of the RNA into the cytoplasm. At the same time that DAF binding turns on Abl kinase, a kinase called Fyn is activated; this promotes viral recruitment to and internalization via caveolar membranes during the entry process. — SMH



Virus (green) in caveolae and CAR (red) at tight junctions.

Cell **124**, 119 (2006).

MATERIALS SCIENCE

Small and Sensitive

Fiber optic systems offer significant bandwidth and efficiency advantages as compared with traditional current-bearing wires. However, shifting the carrier from electrons to photons requires the development of alternative switch and detector technologies. Recently, indium phosphide nanowires were investigated for potential use as integrated detectors in photonic devices and optical switches.

Pettersson *et al.* have prepared more complex heterostructures and analyzed their response across a range of infrared wavelengths. The authors grew indium arsenide (InAs) wires with a core region including either 15 or 35% phosphorus, and then incorporated them into photodetection devices. The energy gap between the InAs and InAsP conduction bands strongly reduced the dark current (that is, the current measured when the wires are not exposed to light), and the spectral response could be modulated by the extent of phosphorus doping. Moreover,

Continued on page 745

CREDITS (TOP TO BOTTOM): JEAN EVERETT/COLLEGE OF CHARLESTON; HUGH WILSON/DIGITAL FLORA OF TEXAS; COYNE AND BERGELSON, *CELL* **124**, 119 (2006)

Continued from page 743

light that was polarized parallel to the wire induced 10 times more current than orthogonally polarized light, a property attributed to the large dielectric contrast between the nanowires and surrounding medium. The results suggest considerable promise for these structures as efficient infrared polarization-sensitive detectors in the 0.65- to 1.4-eV energy range. — MSL

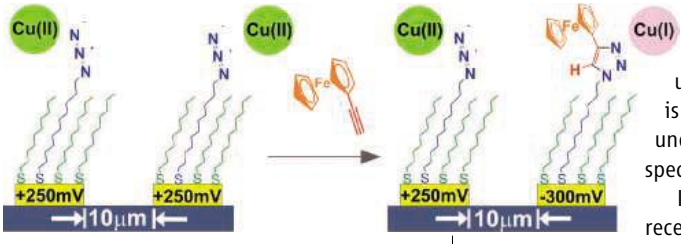
Nano Lett. 10.1021/nl052170l (2006).

CHEMISTRY

Stick, Switch, Click

Microelectrode arrays can be useful in sensor devices, but the application of such arrays depends on being able to modify their surfaces in a controlled fashion. Devaraj *et al.* have adapted a "click" reaction—the high-yield coupling of an azide to an alkyne—so that microelectrodes that are only 10 μm apart can be derivatized sequentially using the same ligation chemistry.

Azide-terminated alkane thiols were self-assembled onto gold microelectrodes on a silicon substrate, and then placed in contact with an electrolyte solution containing a Cu(II) bis(bathophenanthroline)disulfonic acid complex and ethynylferrocene (the alkyne). Switching on a negative bias (0.3 V) at one microelectrode reduced the copper complexes in the immediate vicinity to the active Cu(I) state, which enabled them to catalyze the click



Localized coupling of alkyne (orange) and azide (blue).

reaction between the azide and the alkyne. Nearby, positively biased microelectrodes were not functionalized and remained available for subsequent priming and reaction with other alkynes. — PDS

J. Am. Chem. Soc. 10.1021/ja058380h (2006).

CREDIT: DEVARAJ *ET AL., J. AM. CHEM. SOC.* 10.1021/JA058380H (2006)

ASTROPHYSICS

A Bright Window into the Very Distant Past

Gamma-ray bursts are extremely energetic flashes that are related to the deaths of stars. Their afterglows have been traced as x-rays and in the optical spectrum, which puts constraints on the physical mechanisms responsible for the energetic emission. Their brightness means that they are visible at great distances and hence carry information from long ago.

Using the Swift x-ray telescope, Watson *et al.* have detected the afterglow from the most distant gamma-ray burst yet: GRB 050904, with a redshift of 6.295. Its x-ray emission is highly variable, brightening and dimming on a time scale ranging from a few minutes to half a day. At its height, GRB 050904 was a luminous x-ray source, outshining the brightest quasars at that redshift by a factor of 100,000. Evidence of absorption in its spectrum suggests that oxygen and other elements formed in stars were already widespread in the young universe. This observation indicates that bright and distant gamma-ray bursts, rather than quasars, may be the best background sources for absorption studies of the intergalactic medium within a billion years of the Big Bang. — JB

Astrophys. J. **637**, L69 (2006).

ECOLOGY/EVOLUTION

Making Space for All Types and Sizes

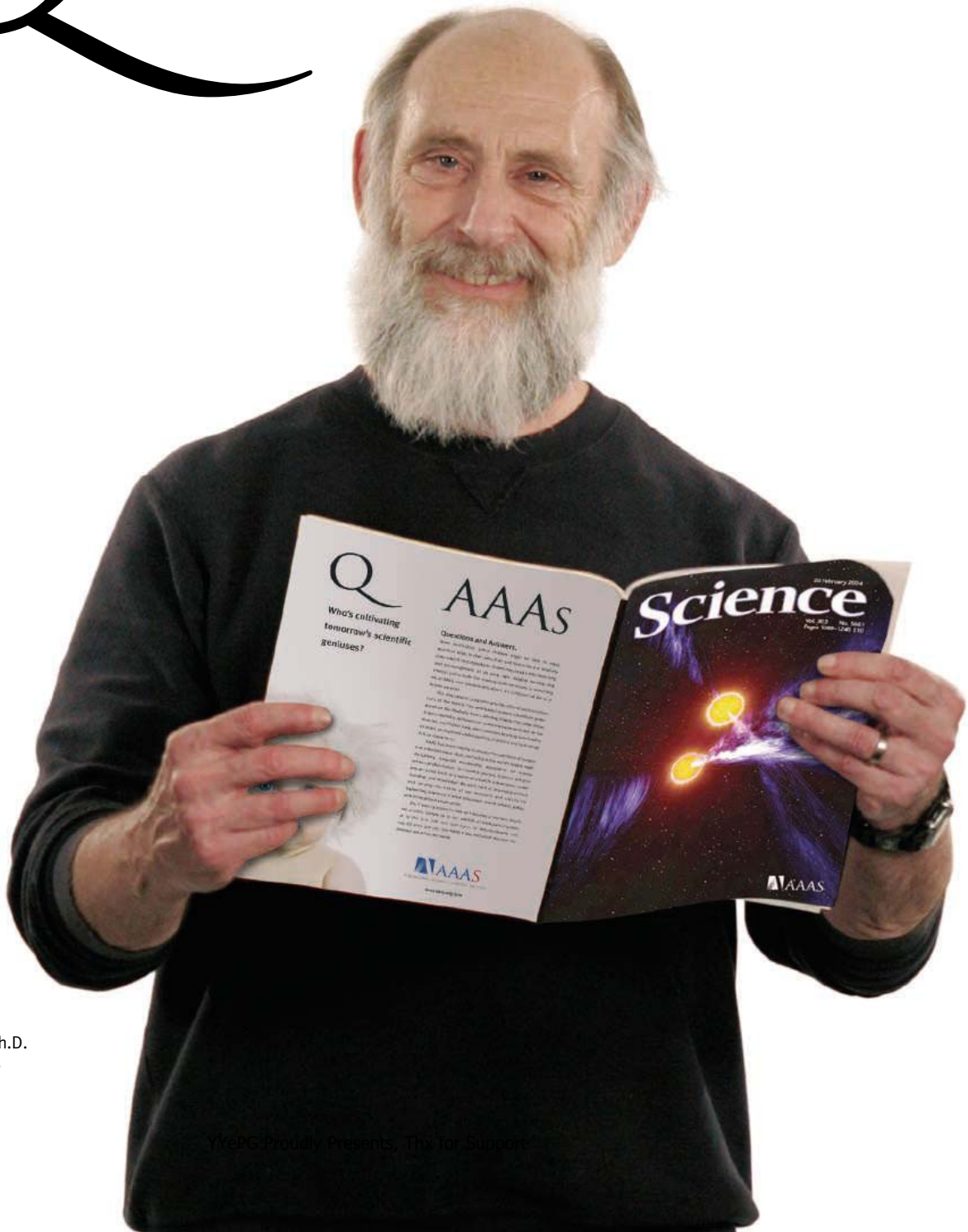
Tree species in tropical rain forests vary widely in their maximum height at adulthood and thus occupy many levels in the forest. In contrast, trees in temperate forests tend to concentrate in the upper canopy, and there is a relative scarcity of understory or subcanopy species.

King *et al.* tested a recent forest dynamics model indicating that greater diversity in adult stature in tropical forests as compared to temperate forests reflects the reduced exclusion of smaller species by canopy species. Measurements of the relative abundances of adult subcanopy species and saplings of canopy species in temperate, subtropical, and tropical forests indicate that there are greater rates of recruitment and establishment of subcanopy species in low-latitude habitats. The underlying mechanism that allows the greater diversity in tree stature in tropical forests may be a combination of varying crown geometries, the length of the growing season, and the extent of light penetration to lower levels in the forest through gaps in the upper canopy. — AMS

J. Ecol. **94**, 1135 (2006).

Q

Who's opening the pipeline to new discoveries?



Leonard Susskind Ph.D.
Professor of Physics
and AAAS member

AAAS

"I started out as a plumber in the Bronx, New York. My father was a plumber. He wanted me to go to college to learn engineering so we could go into business together.

But I was no good at engineering and switched to physics. I got hooked, and quickly knew that I wanted to be a physicist. I had to break it to my father. He didn't know what a physicist was, so I said – like Einstein.

Well, I may not be Einstein but I did become a physicist. It appeals to my curiosity.

At some point I just knew I wanted to spend my life finding out how the natural world works.

I'm a member of AAAS because I believe in what it does for science and scientists. A big part of that work is in education. I think its efforts to bring on the next generation of scientists are vital for our future."

Dr. Leonard Susskind is a professor of physics at Stanford University. He's also a member of AAAS.

See video clips of this story ond others at **www.aaas.org/stories**

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Information for Contributors

See pages 102 and 103 of the 6 January 2006 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

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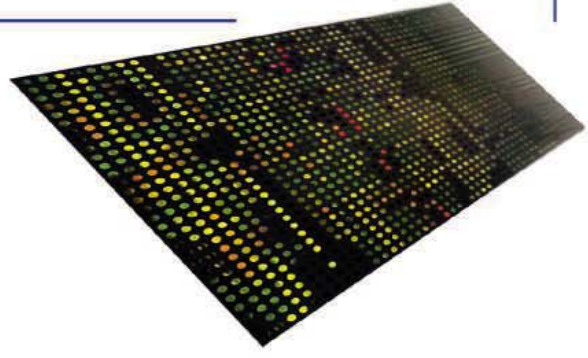

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Mendel at the Vet's >>

A Siamese cat owes its dark ears, paws, and tail to a single mutation in tyrosinase, an enzyme involved in coat color, scientists reported last year. To learn more about inherited traits in animals, many of which serve as models for human diseases, check out the revamped Online Mendelian Inheritance in Animals (OMIA) (NetWatch, 13 December 2002, p. 2097). Curated by Frank Nicholas of the University of Sydney, Australia, the site describes more than 2500 genetic disorders and traits in cats, chickens, cattle, horses, dogs, and 130 other species (except mice). Last September, the U.S. National Center for Biotechnology Information launched an OMIA mirror, integrating the site with GenBank, PubMed, and other NCBI databases. And a new home page in Australia allows guest curators to modify pages. Experts around the world are lining up, says Nicholas. >> omia.angis.org.au

TOOLS

Quick-Change Act

Many Web sites offer conversion programs to transform miles into kilometers, or Celsius into Fahrenheit, but how many can turn becquerels into rads or calculate the wavelength of a photon of energy? Scientists needing such help can call upon the Versatile Unit Converter from Christophe Berthold, a physicist at the University of Geneva. The handy Web tool can convert energy, length, volume, power, temperature, and so on as well as units of radioactivity, electricity, and magnetism. >> mypage.bluewin.ch/berthod/vuc

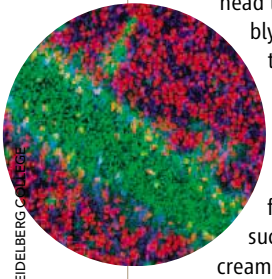
DIRECTORY

Where the Ethicists Are

This new site from the United Nations Educational, Scientific, and Cultural Organization serves as a worldwide catalog of resources on bioethics and the ethics of science and technology. Dubbed the Global Ethics Observatory, the site includes a Who's Who of nearly 500 ethics experts, a list of some 130 ethics organizations, and a smaller directory of courses. A fourth database on ethics legislation and guidelines is coming later this year. >> www.unesco.org/shs/ethics/geobs

EDUCATION

Under the Microscope



If you want to bone up on microscopy techniques or need images for a biology class, head to Molecular Expressions, probably the largest microscopy site on the Internet. Run by Michael Davidson at the National High Magnetic Field Laboratory in Tallahassee, Florida, the site became popular a decade ago for its close-ups of everyday items such as electronic circuits and ice cream. It's now a sprawling collection of educational resources.

Researchers may want to head to the Optical Microscopy Primer, which offers simulators of various microscopes and tutorials on their use. The related MicroscopyU site, produced with Nikon, includes new movies of live cells crawling and splitting that many professors use in classes, says Davidson. K–12 teaching resources include a cell biology primer and a "Powers of 10" applet that zooms from space into the cells of an oak leaf. Check out early microscope designs, or read up on optics luminaries such as Holland's Antonie van Leeuwenhoek (1632–1723), the first scientist to see bacteria. The site's galleries can be dazzling. Above, a slice of rat brain tissue. >> micro.magnet.fsu.edu

CREDITS (TOP TO BOTTOM): GETTY IMAGES; M. DAVIDSON/FLORIDA STATE UNIVERSITY; D. BAKER/HEIDELBERG COLLEGE

DATABASE

READING THE RIVERS

In 1974, amid growing concern about pollution in the Great Lakes, researchers at Heidelberg College in Tiffin, Ohio, began tracking the stream chemistry of the state's rivers. Their work quantified watershed pollutants from sources such as sewage plants and rural runoff, and it led to efforts to stem the flow of agricultural phosphorus into Lake Erie. At this new site, project leader David Baker, now a professor emeritus, and colleagues share their wealth of data on 11 rivers for scientists to use in courses or research. Visitors can download Excel files for more than 88,000 water samples tested for phosphorus, nitrates, suspended solids, and other components. Tutorials put the information in context, and templates help users analyze the data. Above, sediment disgorged by a flooding Sandusky River drifts into Lake Erie. >> wqldata.heidelberg.edu

Send site suggestions to NETWATCH@aaas.org. **Archive:** www.sciencemag.org/netwatch

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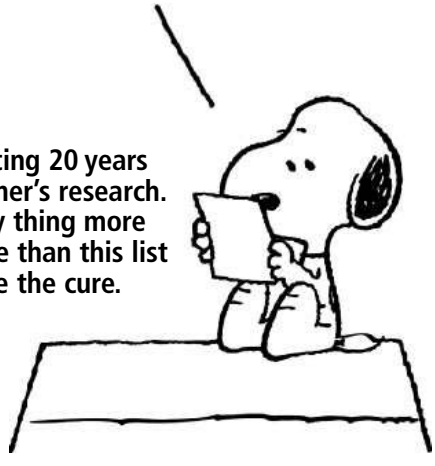

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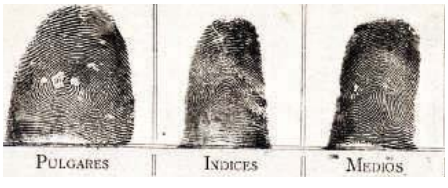
NEW ROO FIND

This golden-mantled tree kangaroo, killed by indigenous subsistence hunters, was one of the surprise finds from a biodiversity survey conducted in December on the western, or Indonesian, half of New Guinea. The species was thought to inhabit only a single area in eastern New Guinea and had only been recorded once before by scientists, in 1988, says Bruce Beehler of Conservation International (CI) in Washington, D.C.

The "rapid assessment survey" by CI scientists was the first attempt to document flora and fauna in the Foja Mountains' vast tract of unspoiled rainforest. The group cataloged an array of treasures, including a new honeyeater, a "lost" bird of paradise, dozens of new frog and butterfly species, and giant rhododendrons.

DIGITAL PROOF

The fingerprints below are from an Argentinian murderer, Francisca Rojas, who in 1892 became the first person to be convicted based on such evidence. The card is part of a new exhibit, "Visible Proofs," tracing the history of forensics that will open on 17 February at the National Library of Medicine in Bethesda, Maryland. Among its curiosities are the instruments used for President Lincoln's autopsy, a human heart pierced by a bullet, early medical treatises, and film clips of autopsies.



Swelled Heads

An unusual study of old skulls has revealed that human heads have gotten significantly bigger than they were just a few centuries ago.

Led by orthodontist Peter Rock of the University of Birmingham, U.K., researchers measured 30 skulls from male and female victims of London's Black Death epidemic of 1348–'49 and 54 male skulls brought up from a warship, the *Mary Rose*, which sank in England's Portsmouth harbor in 1545. The team compared the old skulls with x-rays from 31 modern young adults of both sexes. The height of the modern group's cranial vaults exceeded that of both historic samples by about 15%, the team reported last month in the *British Dental Journal*. Although it is well known that body size has increased over the centuries as diets have improved, Rock says he found hints that brain size might have increased independently: Faces have become less prominent in relation to foreheads over the centuries, he says, and the part of the skull that holds the brain's frontal lobes—the part associated with intelligence—was proportionally larger in modern skulls.

Skull from Black Death.

"I think it's a very exciting study because they have two very interesting samples from the past," says primatologist Robert Martin of the Field Museum in Chicago. But the significance of the brain change can't be determined, especially because there are no bones available to reveal the relationship to body size.

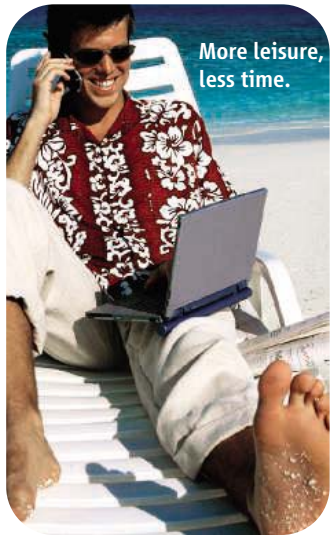
BUSY RELAXING >>

Americans may always be complaining about having too much to do, but the fact is they have enjoyed a phenomenal increase in leisure time over the past 40 years, according to two economists.

Based on surveys of self-reported time use through the decades, Erik Hurst of the University of Chicago reported last week that those in the U.S. job market had 6 to 8 hours more leisure time per week in 2003 than they did in 1965. Total hours devoted to leisure in the narrowest sense—activities pursued solely for enjoyment—rose from 31.04 to 35.65 a week. Speaking at the American Enterprise Institute in Washington, D.C., Hurst also said that although 74% of women were in the job market in 2003—compared with 48% in 1965—leisure time for both men and women in the 21-to-65 age range had increased "dramatically."

While hours worked per week have remained stable, there's been a huge time savings—about 12 hours a week—in housework, according to Hurst. TV watching is devouring two-thirds of the time gained, while time spent reading and churchgoing is down.

Economist Valerie Ramey of the University of California, San Diego, who reported on data from the entire U.S. population, said that there's a "higher fraction of harried people now," which may have led to a "myth" that people are working more. In reality, "we're doing more things," she said. Both economists predicted that leisure time will continue to increase, because people are living longer and have fewer children to care for.



More leisure, less time.

CREDITS (TOP TO BOTTOM): CONSERVATION INTERNATIONAL; UNIVERSITY OF BIRMINGHAM; DIRECCIÓN MUSEO POLICIAL, BUENOS AIRES, ARGENTINA; ROYALTY FREE/CORBIS

Anxiety changes reward pathway 759

Logging paper generates a firestorm 761

SCIENTIFIC MISCONDUCT

Investigations Document Still More Problems for Stem Cell Researchers

SEOUL—The accusations surrounding Woo Suk Hwang's discredited stem cell research have gone from bad to worse. Last week, a report from the South Korean National Bioethics Committee said that Hwang and his team seriously violated basic ethical rules in their collection of human oocytes and that some of the 119 donors became severely ill as a result of the procedure. The government's auditor also said on Monday that it so far could not account for $2.6 million in research funds that Hwang had received. And there could be more to come: At least five investigations are continuing in South Korea and the United States.

Warm reception. Jong Hyuk Park, a member of Hwang's team, is mobbed by reporters when he returned to Seoul last week from the United States to talk to prosecutors.

CREDIT: YONHAP NEWS AGENCY

The initial results of the audit have been referred to South Korean prosecutors, who are investigating potentially criminal aspects of the saga. Meanwhile, investigations are under way at *Science*, which published both of Hwang's now-discredited papers claiming to have derived embryonic stem cells from cloned human embryos, and at two U.S. universities where Hwang co-authors work.

On 6 February, the South Korean government's auditor said in a report that Hwang could not account for how he spent a significant sum of his research money, which included $31.8 million (30.9 billion won) in public funds and $6.2 million (6 billion won) from private sources. The Bureau of Audit and Inspection said Hwang could not prove how he used $1.07 million from the state and $1.6 million in private funds. Hwang also deposited public and private funds into his personal account and withdrew money for purposes "outside of research," the report says, although auditors do not know exactly how the funds were spent.

Some apparently went to lab members involved in the scandal. Shortly after questions were raised last fall about how Hwang obtained oocytes, news media reported that two of his co-authors who were working at the University of Pittsburgh, Pennsylvania, Jong Hyuk Park and Sun Jong Kim, together received a total of $50,000 from Hwang's associates. (Seoul National University officials said in December that Kim turned over $30,000 that he had been given.) The auditors say this money came from the funds Hwang received from private sources.

In a separate investigation, the National Bioethics Committee said in an interim report released 2 February that Hwang's team received at least 2221 oocytes from 119 women between November 2002 and December 2005, 160 more than Seoul National University reported last month. (In their papers, Hwang and his colleagues reported using only 427 oocytes.) Citing "serious ethical violations," the panel also found that Hwang's team failed to fully explain the potential risks associated with oocyte donation and that the Institutional Review Boards at Hanyang University's medical center and Seoul National University provided insufficient oversight.

The panel says that a significant number of women who donated through MizMedi Hospital developed ovarian hyperstimulation syndrome, a side effect of the drugs given to oocyte donors. Fifteen out of the 79 MizMedi donors were treated for the syndrome, which can cause nausea in mild cases and liver and kidney damage in severe cases. The committee said two donors were hospitalized. The report also said that some women who suffered from health effects went on to donate again despite the risks.

Among the 119 donors, 66 received compensation. The committee said it is still looking into whether any of the payments occurred after 1 January 2005, when a law went into effect prohibiting such payments.

That is one of the questions the Seoul Central District Prosecutors' Office is trying to answer as a special team questions key figures associated with Hwang's fabricated research. As prosecutors try to pinpoint who did what in the labs, they are also looking into whether Hwang misused public funds and whether someone at MizMedi Hospital, which collected oocytes for his research, switched his cloned embryonic stem cells with fertilized ones, as Hwang contends. The prosecutors continue to interview lab members, and they raided Hwang's home for a second time last week. They have also asked University of Pittsburgh professor and co-author Gerald Schatten to travel to South Korea for questioning. University spokesperson Jane Duffield said Schatten would seek legal advice on how to respond. She said the university's own investigation was likely to finish in mid-February.

Sung Il Roh, director of MizMedi Hospital, told *Science* that he expects to talk to the prosecutors by next week. Jong Hyuk Park and Sun Jong Kim have already been questioned, and prosecutors are expected to call co-author and former MizMedi researcher Hyun Soo Yoon, now a professor at Hanyang University.

The revelations about oocyte donations have triggered the retraction of yet another paper associated with Hwang's work (*Science*, 20 January, p. 321). On 31 January, the *American Journal of Bioethics* announced that it is ▶

retracting a paper about ethics and egg donation that appears in its January-February issue. The article, by ethics and legal expert Koo Won Jung of Hanyang University and bioethicist Insoo Hyun of Case Western Reserve University in Cleveland, Ohio, is based in part on visits to Hwang's lab last summer. Hyun says the article, which first appeared online in November, is being withdrawn because it contains descriptions of lab practices that it is now clear were not followed.

Jose Cibelli, who was a co-author on Hwang's 2004 paper, has also requested that Michigan State University investigate his role in the work.

Science will be conducting an internal review this month, and an external review led by outside scientists will take place in March and report its findings in April. John Brauman, a chemist at Stanford University in Palo Alto, California, and chair of *Science*'s senior editorial board, will head the external panel, which will examine both how the Hwang papers were handled and *Science*'s policies in general. "They will be given whatever they want," says Monica Bradford, *Science*'s executive editor.

–SEI CHONG

Sei Chong is a freelance writer in Seoul. With reporting by Jennifer Couzin, Constance Holden, and Gretchen Vogel.

WOMEN'S HEALTH

Study Yields Murky Signals on Low-Fat Diets and Disease

An 8-year study of nearly 49,000 postmenopausal women that explored links between a low-fat diet and health is leaving confusion in its wake. The study, run by the Women's Health Initiative (WHI), found that individuals asked to adhere to a low-fat diet had roughly the same risk of breast cancer, colorectal cancer, and cardiovascular disease as those whose diet didn't change. But methodological problems have left researchers stymied about what the message of the three-pronged study, published this week in the *Journal of the American Medical Association*, should be. "We have a very sobering situation," says Harvard University epidemiologist Walter Willett. While praising the dedication of WHI investigators, he notes that "this was the biggest and most expensive [diet] study ever done," and it arrived at "a very crude result."

Women's Health Initiative Study

% calories from fat

	Dieters	Nondieters
Original study goal	20%	40%
At 1 year	24%	35%
At 6 years	30%	38%

SOURCE: ROSS PRENTICE, *JAMA* **295**, 632 (2006); (IMAGE) PHOTOS.COM

The study is the second of three from the WHI (*Science*, 10 June 2005, p. 1570). The first, whose results were reported in 2002 and 2004, was controversial. It found that hormone replacement therapy could raise the risk of breast cancer and heart disease, prompting a stampede away from the drugs. The third, examining the effects of calcium and vitamin D on bone health, will be published next week.

The diet study randomized more than 19,000 women to a diet low in fat and high in fruits, vegetables, and grains. A comparison group included 29,000 others. It was hoped that the first group could slash its fat intake to 20% of calories, while the second would hover around 40%. Study leaders predicted that even if the difference in fat intake was just 11% at the study's end, they would see 14% fewer cases of breast cancer among the dieters. The study also examined whether the low-fat diet could avert colorectal cancer and cardiovascular disease.

But, as is common in nutrition studies, participants had difficulty sticking to the diet. After 6 years, dieters were consuming 30% of their calories from fat, compared with 38% in the control group. There was no difference in colorectal cancer or cardiovascular disease rates. Dieters did suffer 9% fewer cases of breast cancer, but that result failed, just barely, to reach statistical significance, meaning it could have occurred by chance. Still, "I don't think it can be dismissed," says Lynn Rosenberg of Boston University School of Public Health.

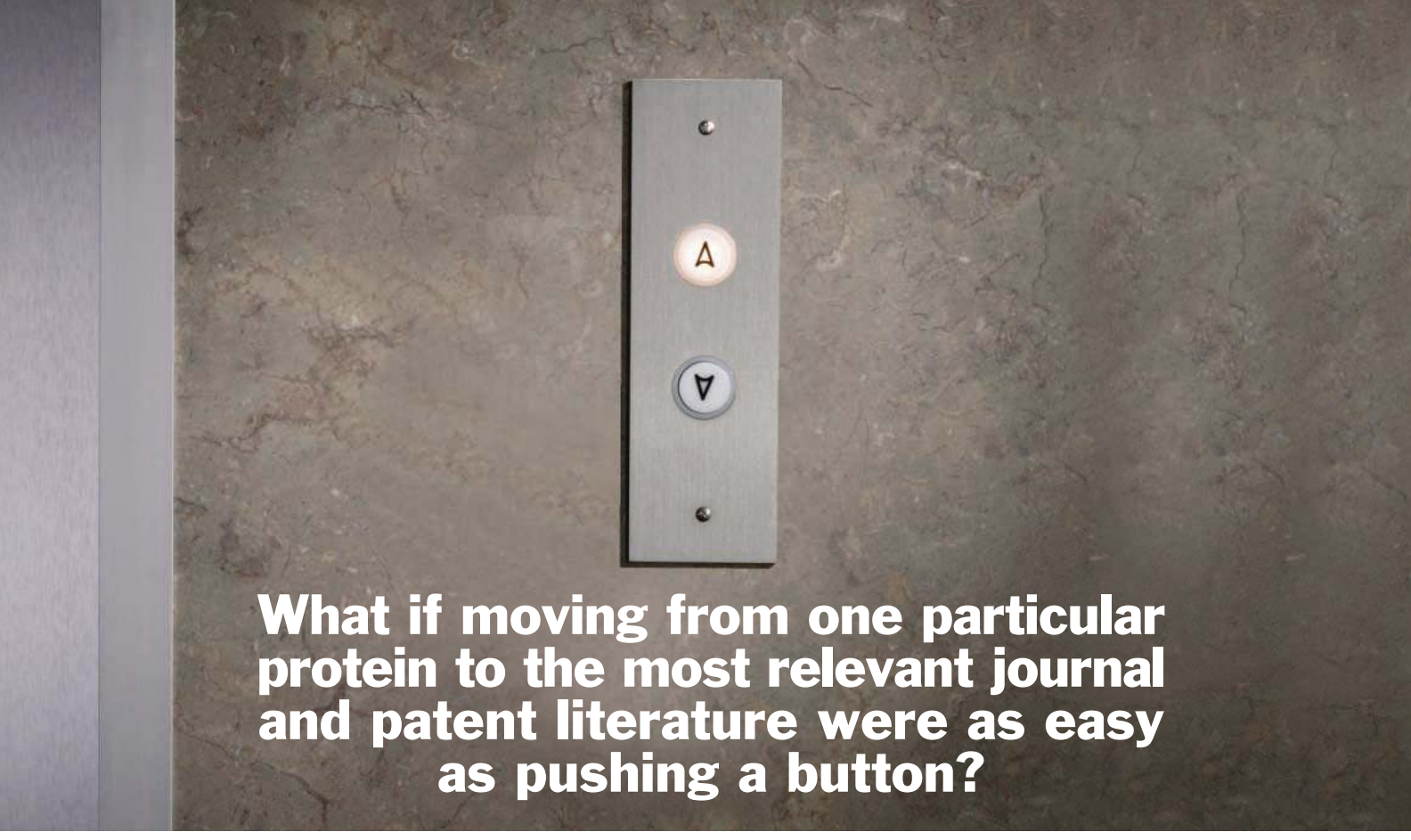
The study's diet was designed with breast cancer in mind, says Ross Prentice, a biostatistician at Fred Hutchinson Cancer Research Center in Seattle, Washington, and a leader of the WHI trial. Although cardiovascular disease can be prevented by replacing saturated fats with polyunsaturated ones, "for breast cancer, it's unclear whether targeting certain types of fat would be a more effective approach," says JoAnn Manson, a WHI principal investigator and chief of preventive medicine at Harvard's Brigham and Women's Hospital in Boston.

In addition to dietary adherence, the study may have been limited by its length, says Willett. Although impressive by most standards, 8 years is relatively brief where diet's effects on slow-growing cancers are concerned. The results could also have been influenced by the fact that participants started the diet late in life: Researchers don't yet know whether diets begun earlier are more powerful than those begun at older ages.

Norman Boyd, a cancer epidemiologist at Princess Margaret Hospital in Toronto, Canada, notes that diet data were collected through food-frequency questionnaires; they were given to participants at the study's launch, after the first year, and every 3 years thereafter. Such questionnaires rely heavily on memory and are "not a very good way of addressing diet," says Boyd. He's finishing a breast cancer prevention study of 4700 women that also tests a low-fat diet followed for at least 8 years. His participants are at risk of the disease and also younger—their average age is 42. Results of Boyd's trial are expected later this year.

Despite the WHI study's mixed results, critics and supporters alike agree that when it comes to disease, diet matters. Although its dieters can now hop off the low-fat bandwagon, WHI investigators will follow them for another 5 years, searching for additional clues about fat's role in health.

–JENNIFER COUZIN



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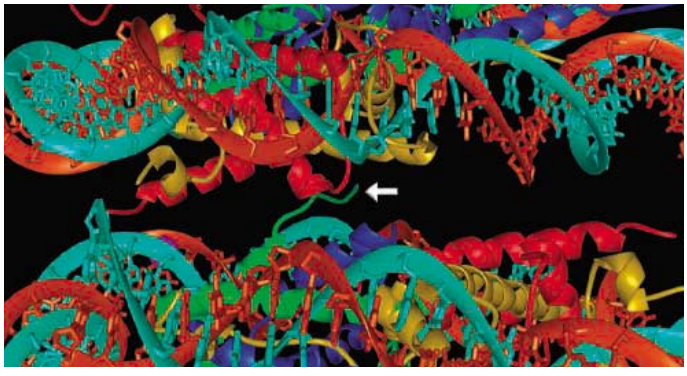
MOLECULAR BIOLOGY

Protein Tail Modification Opens Way for Gene Activity

Turning on a gene is a lot more complicated than simply flipping a switch. Oftentimes, the gene is effectively trapped in chromatin, the complex of DNA and histone proteins that makes up a cell's chromosomes, and thus hidden from the transcription factors needed to activate its expression. New results have now identified a critical histone modification that opens up chromatin so that gene expression can take place.

When not condensed, chromatin looks much like a string of beads with DNA as string and the beads, known as nucleosomes, consisting of DNA wound around a core of histones. In its condensed state, chromatin folds so that the nucleosomes are stacked on one another, a structure that can keep enclosed genes from being expressed.



Making contact. The tail of histone 4 (arrow) can link one nucleosome to another, but not when blocked by acetylation of a specific amino acid on the tail.

On page 844, a team led by Craig Peterson of the University of Massachusetts Medical School in Worcester reports that addition of a single acetyl group to a specific lysine located in the tail of so-called histone 4 (H4) can prevent this folding, presumably by blocking the necessary nucleosome-to-nucleosome interactions. Chromatin researcher Michael Grunstein of the David Geffen Medical School at the University of California, Los Angeles, describes the finding as "central to understanding gene activation. The acetylation renders the entire chromatin open for gene activity."

Acetylation of the histone 4 tail region had previously been implicated in chromatin compaction, but the details remained murky. In 1997, for example, Timothy Richmond's team at Eidgenössicsche Technische Hochschule (ETH) Institute for Molecular Biology and Biophysics in Zurich, Switzerland, determined the x-ray crystallographic structure of the nucleosome. "You can see this region of the [H4] tail interact with adjacent nucleosomes," Richmond says.That suggested it helps tie chromatin into its folded form, a supposition buttressed 3 years ago when the ETH group showed that compaction can't occur if the tail segment is deleted.

Histone acetylation is one part of the so-called histone code: various modifications of these proteins that have been shown to influence gene activity. The acetyl groups attach to different amino acids within the H4 tail, however, and researchers have been unable to pin down what the various additions do, mainly because they have been unable to produce nucleosomes bearing only one particular histone modification.

To try to solve this problem, Michael Shogren-Knaak, a postdoc in the Peterson lab, about 3 years ago developed a technique that produces nucleosomes with specific modifications. This involves first chemically synthesizing the 22 amino acid H4 tail peptide with the desired modification. In the current work, Shogren-Knaak and his colleagues chose to add an acetyl group to the tail's 16th amino acid, a lysine, because it's among the amino acids commonly found acetylated in living cells. The researchers then attached the modified tail to the remaining segment of the H4 protein, which they generated with recombinant DNA technology. Mixing this modified histone with recombinant versions of the other three histones found in nucleosomes and then with DNA generated stretches of chromatin containing 12 nucleosomes, all with the exact same H4 modification.

Adding magnesium salts to nucleosomes normally causes them to compact, but chromatin containing H4 tails with the acetylated lysine failed to fold when treated with the salts. Those modified nucleosomes "were stuck as 'beads on a string,' " says Peterson. This is "the first time," notes Richmond, that someone has shown that a specific histone modification dramatically changes the state of chromatin.

Although the complex histone code governing gene activity continues to mystify, scientists seem to have cracked at least one of its secrets.

YYePG Proudly Presents, Thx for Stopping! **–JENNIFER MARX**

CREDIT: ADAPTED FROM C. A. DAVEY *ET AL.*, *JOURNAL OF MOLECULAR BIOLOGY* **202**, 1097 (2002)

Biosafety Building Gets NIH Nod

Despite vociferous opposition from neighborhood groups, Boston University (BU) will soon begin construction on a biosafety level 4 (BSL-4) lab at its medical campus in the city's South End. The National Institutes of Health (NIH), which is funding most of the $178 million project, granted final approval last week after completing review of BU's environmental impact study. Critics argued that the lab, which will handle the most dangerous bacteria and viruses, should not be built in an urban center. But NIH determined that BU's safety procedures were adequate.

The Roxbury, Massachusetts–based nonprofit Alternatives for Community and Environment, which has led the fight against the lab, says that NIH did not sufficiently consider alternative sites. The group is pressuring the city council to make it illegal to build a BSL-4 lab within the city limits. That effort, however, remains stalled. **–ANDREW LAWLER**

Brave Nuclear World

Rekindling the Atoms for Peace spirit of the 1950s, the U.S. Department of Energy (DOE) this week rolled out a Global Nuclear Energy Partnership (GNEP) that it hopes will facilitate "a nuclear renaissance" worldwide. Critics say the scheme, which seeks to lease reprocessed nuclear fuel to friendly nations, will be too expensive and could heighten proliferation risks (*Science*, 2 December 2005, p. 1406). Spending on U.S. nuclear fuel cycle research, a large part of the program, will more than double in the 2007 budget proposal to $250 million.

GNEP would develop ways to reprocess spent fuel from existing reactors rather than socking it straight away in a repository, such as the long-planned Yucca Mountain facility in Nevada. Decades of reprocessing abroad have accumulated about 200 metric tons of plutonium. The United States rejected reprocessing in 1970, but officials say global energy needs and promising science have driven the turnaround. GNEP aims to develop a fuel laden with radioactive actinides that is "not attractive or usable as weapons material," DOE's Clay Sell told reporters this week. The leased fuel would be monitored and returned after use, echoing Russia's recent proposal to lend fuel to Iran.

Although GNEP's objectives are "laudable," says Harvard nonproliferation expert Matthew Bunn, reprocessing "would cost tens of billions of dollars" in the near term and "involve significant risks." He prefers storing spent fuel in dry casks. DOE plans to deliver legislation to Congress later this month.

–RICHARD STONE AND ELI KINTISCH

TECTONICS

An Early Date for Raising the Roof of the World

Knowing when something first appeared on Earth can tell much about how and why it appeared. So researchers were keen this week to hear evidence that the Tibetan Plateau already towered over the rest of the planet 35 million years ago. That's tens of millions of years earlier than previous data suggested and not long after India first smashed into that part of the world.

Early Tibet, early to rise. Isotopes in rocks exposed on the central Tibetan Plateau suggest it rose early and high.

The proposed timing suggests to plate-tectonics specialists that India, by shoving itself into Asia, raised the plateau to its extraordinary 5000-meter altitude—higher on average than the highest peak in Europe or the contiguous United States. It also suggests that the Tibetan Plateau was sticking up into the atmosphere far earlier than thought, redirecting global winds, stoking the monsoon, and perhaps weakening the greenhouse.

Signs of kilometer-scale plateau growth come in atom-by-atom measurements reported this week in *Nature* by David Rowley of the University of Chicago and Brian Currie of Miami University in Oxford, Ohio, both working in the young field of paleo-altimetry. Rowley, a tectonicist and field geologist, and Currie, a geochemist, measured the oxygen isotopic composition of carbonate minerals from the Lunpola Basin, near the center of the plateau, that were deposited on lake bottoms or formed in soils.

The more abundant the light oxygen isotope relative to the heavy isotope, the higher the elevation of the spot where the carbonate formed. That's because as water vapor–laden air climbs to higher and higher altitudes, water molecules carrying heavy oxygen preferentially fall out as precipitation, leaving the remaining water vapor isotopically lighter. Once the water falls as precipitation on the plateau, it passes that isotopic signature of elevation gain on to carbonates as they form. Last year in the journal *Geology*, the researchers reported that applying the technique to 15-million-year-old carbonates from a basin south of Lunpola matched earlier elevation estimates that researchers had made by analyzing the shapes of fossil leaves.

This time, Rowley and Currie found that 35 million years ago and about 20 million years ago, the ancient Lunpola Basin stood about 4 kilometers above sea level—almost as high as it does today. A high central plateau 35 million years ago would mean that the northward-moving Indian continent plowed into the Asian continent like a bulldozer, thickening the Asian crust. Because continental crust is more buoyant than underlying mantle rock, that would have floated the plateau higher. The plateau would also have been buoyed up in a rival scenario: The denser mantle rock that makes up the lower part of a tectonic plate formed a blob, detached itself from Asia, and fell away into the deep mantle—but not until perhaps 10 million years ago.

"They've got an interesting story," says Carmala Garzione of the University of Rochester in New York, who also uses carbonate isotopes for paleo-altimetry. "The technique is robust," Garzione says, although Rowley and Currie's calibration method differs from hers. "The theory's fine," agrees tectonicist Peter Molnar of the University of Colorado, Boulder, "but is it applicable" to 35 million years ago? Water might have taken a different route to the plateau then, he says, shedding heavy isotopes more or less efficiently than in the present day and throwing off their paleo-elevation calculation.

"Paleo-altimetry is incredibly cool," concludes tectonicist Leigh Royden of the Massachusetts Institute of Technology in Cambridge, "but it's still in its infancy. We don't have enough data to say one way or the other, [but] we're going to know in 10 years."

–RICHARD A. KERR

ASTROPHYSICS

Dwarf Galaxies May Help Define Dark Matter

CAMBRIDGE, U.K.—Researchers here say they have found the first physical properties of dark matter, the invisible stuff that makes up most of the substance of the universe. In research that is yet to be written up—let alone published—a team led by Gerry Gilmore of the Institute of Astronomy at Cambridge University saw a common feature in dwarf galaxies that are satellites of our own Milky Way galaxy: They all had a core of dark matter of a uniform size and temperature—somewhat warmer than the most popular theories of dark matter predict. Gilmore suggests this could be "an intrinsic property of dark matter." The claim alone "will generate a lot of excitement," says cosmologist Robert Nichol of the University of Portsmouth, U.K.

Second fiddle. The new results show that our Milky Way is actually more massive than Andromeda (pictured).

The new results came out almost by accident. On 3 February, Gilmore appeared with others at a press conference in London to publicize the work of the European Southern Observatory (ESO). Gilmore described his results, which used some of the world's largest telescopes including ESO's Very Large Telescope in Chile, to argue that even better telescopes would be needed to take the research further. But the assembled journalists ▶

CREDITS (TOP TO BOTTOM): ANDREW CYR, COURTESY OF BRIAN CURRIE; NASA/JPL

found Gilmore's research far more interesting; several national dailies carried news of the results on Monday morning. Gilmore says a paper is "partially written up."

For the past 3 years, Gilmore and his team have been using giant scopes to map the positions and velocities of thousands of stars in 10 minigalaxies around the Milky Way, working out a three-dimensional mass distribution for each. Astronomers have known for decades that the mass of visible stars doesn't provide enough gravity to hold galaxies together. They concluded that large amounts of dark matter must make up the balance. But they've been stumped in their efforts to locate or describe it. Now the Cambridge team says it has found a uniform volume of dark matter in each galaxy, about 1000 light-years across and with a density equivalent to four hydrogen atoms per cubic centimeter.

The most popular theory suggests that dark matter is made up of massive exotic particles that do not interact with normal matter except through gravity. It also holds that the particles would have low velocities and low temperatures. This model fits the structure of most galaxies and large-scale structures in the universe. But elsewhere it falls down, predicting many more small galaxies than we actually see and a high-density "cusp" of dark matter surrounded by fast-moving stars at the center of small galaxies—also not seen.

The new results suggest that the dark matter at the center of small galaxies is more spread out and warmer than prevailing theories predict. The particles appear to have a velocity of 9 kilometers per second. Gilmore suggests that they interact with one another to spread out evenly. "There must be some form of repulsion," he says, adding "this is the first clue of a property of dark matter."

Other researchers are, understandably, viewing the results with caution, not having seen details of the observations or Gilmore's interpretation. "If correct, it is a good argument for warm dark matter. It would rule out many of the most popular ideas," says David Weinberg of Ohio State University, Columbus. But he is skeptical about chargelike repulsion among the dark matter particles in small galaxies because such approaches have "some pretty severe problems" when applied to other galaxy types. Mario Mateo of the University of Michigan, Ann Arbor, who also studies dwarf galaxies, says the results "sound interesting," although he is surprised by the density of dark matter Gilmore found. He says it's "pretty amazing" that although scientists can't see it or measure it, "we can start talking about constraining the nature of dark matter."

The Cambridge study also produced a mass for the Milky Way, revealing that it is not lighter than Andromeda but is top dog in our local group.

–DANIEL CLERY

Cowed mouse. After being bullied by a bigger mouse, mice experience brain changes that increase their fear of unfamiliar mice.

CREDIT: O. BERTON *ET AL.*, *SCIENCE*

NEUROSCIENCE

Bullied Mice Implicate Brain's Reward Pathway in Mood Disorders

Chronic bullying and intimidation can make a person—or a mouse—fearful and withdrawn. Now scientists have shown in bullied mice that the brain's reward circuits—the areas usually associated with addiction—play a big role in these reactions. Furthermore, they find that such negative responses are enabled by brain-derived neurotrophic factor (BDNF), a chemical that elsewhere in the brain is associated with antidepressant actions.

On page 864, a team led by neuroscientist Olivier Berton at the University of Texas Southwestern Medical Center in Dallas reports the results of experiments in which they exposed individual mice to a different, big bully mouse every day for 10 days, creating strongly aversive behaviors in the victims. Unlike typical mice, the cowed mice act frightened even when caged with an unfamiliar, nonbully mouse. The changes were long-lasting: The "defeated" mice maintained their phobic reactions even 4 weeks after exposure to the aggressors.

Subsequent experiments showed that the bullied, fearful mice had an altered mesolimbic dopamine system, the brain pathway best known for reinforcing addictive behavior through the release of dopamine. "This social-defeat process induced [production of] BDNF in the reward circuit," says the senior author, psychiatrist Eric Nestler.

BDNF stimulates nerve cell growth, and it is hypothesized that some antidepressants work by boosting BDNF production, leading to the growth of new neurons in the hippocampus. But Nestler's team found that in the areas comprising the reward circuit, the bullying-induced BDNF facilitates long-term neuronal changes that cause the development of social aversion, a common symptom of depression. When they injected mice with a virus that knocks out BDNF production solely in this circuit, the mice were no longer intimidated by the bullies.

Although the reward neural circuit is of intense interest, Nestler's group concludes that it also plays a part in depression, social phobias, and even posttraumatic stress disorder. "This paper for the first time establishes an important role for BDNF in a brain circuit that clearly is involved in a host of devastating neuropsychiatric disorders" besides addiction, says psychiatrist Robert Malenka of Stanford University in Palo Alto, California.

Malenka notes that the brain's reward system has been slighted in research on emotional disorders even though "it's kind of intuitive" that those pathways would also be involved in depression, because inability to experience any rewarding feelings is a hallmark of depression. This work, he says, "puts BDNF in the dopamine system front and center" in disorders involving emotional withdrawal.

In the hippocampus, BDNF is associated with learning and memory. With the new study, says Nestler, the chemical is now "implicated in a different nerve circuit, playing a role in a different type of learning: [long-term] social learning." Harvard psychiatrist Steven Hyman, a former director of the National Institute of Mental Health, points out that this is another instance of brain chemicals' functions being dependent on their location. And by highlighting the importance of BDNF and the mesolimbic dopamine system, he says, the new study is "one more salient reminder that reward systems, too long neglected, are likely to play a critical role in mood regulation."

Hyman agrees with Nestler that the mouse study suggests that the brain's reward circuitry is a new target for drugs treating human mood disorders. The results also indicate that the stress from chronic anxiety may be treatable by antidepressants, says Nestler. He notes that although tranquilizers normally help alleviate acute anxiety, benzodiazepine had no effect on the bullied mice, whereas fluoxetine (Prozac) had the same effect as deleting BDNF.

–CONSTANCE HOLDEN

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Scorched Earth. Controversy remains over a paper that measured harm done by postfire logging.

ECOLOGY

Salvage Logging Research Continues To Generate Sparks

A premier forestry department is still smoldering over a controversial paper about salvage logging. The research garnered national headlines in early January when *Science* published a paper online by researchers, including some from Oregon State University (OSU), who concluded that logging after wildfires hinders the regeneration of forests and increases the risk of further fires. The paper made headlines again a few days later when another group of OSU faculty members asked that print publication be delayed until their criticisms were addressed.

That request led to cries of attempted censorship. The group of critics, in turn, charged that the paper was politically motivated. Now, a government agency that helped fund the study has put a hold on the grant, pending an investigation. "I expected a dustup, but nothing of this scale," says Jerry Franklin of the University of Washington, Seattle, who says he reviewed the paper.

Salvage logging is a long-standing forestry practice. If a wildfire kills trees but doesn't completely burn them, logging companies will harvest those logs and plant tree seedlings. Proponents of the practice say it can accelerate forest regrowth and make forests safer for firefighters.

Environmentalists have long criticized the practice, however, charging that logging machinery tears up the soil and that hauling out the dead wood removes valuable habitat for wildlife. But not much research has been published in peer-reviewed journals on the effects of postfire salvage logging. The topic is hot now because two bills pending in Congress would make it easier for companies to do salvage logging in national forests.

Enter the *Science* paper. The research comes from an ongoing study of the 2002 Biscuit Fire, which ravaged 200,000 hectares in southern Oregon. Comparing plots before and after salvage logging finished last year, a team led by plant physiologist Bev Law of OSU Corvallis and Boone Kauffman of the U.S. Forest Service (USFS) found 71% fewer naturally sprouted seedlings in the logged plots. Much of the work was done by first author Dan Donato, a second-year graduate student. Downed branches and twigs left over from the logging increased the amount of flammable material on the forest floor by severalfold, compared to the burned but unlogged plots. The team concluded that salvage logging hinders forest recovery and actually exacerbates fire risk—a finding that contradicts assertions made on behalf of the practice.

What really stirred up controversy, however, was a letter sent to the *Science* editors on 17 January by John Sessions, a forest modeler at OSU. He and eight co-authors from the university and USFS pointed out what they considered to be serious shortcomings in the paper. They say the conclusions are preliminary and that the paper didn't put the findings into context—neglecting to describe soil moisture at the site, for example, and not spelling out that fire risk is more complex than just the amount of dead wood left behind: "We believe that the peer review process failed." The letter was reported by *The Oregonian*, the state's largest newspaper.

Their request to delay publication of the print version of the paper until these concerns were corrected, or to print them alongside the paper, struck some as meddling with peer review. "I was stunned," says OSU's Barbara Bond. The paper appeared in print on schedule (*Science*, 20 January, p. 352). "We have confidence in our peer review decision," says *Science* Editor-in-Chief Don Kennedy. "I think it's fairly clear [the letter] was an effort to suppress a paper."

The critics deny that and charge in turn that the authors of the *Science* paper are attempting to sway the debate on the bills in Congress. Sessions points out that the online version of the paper referred to the House and Senate bills, and the Bureau of Land Management (BLM) is now investigating whether this crossed the line of using government funds for lobbying.

Two other facts make critics suspect politicking. One of the administrators of the grant, former BLM ecologist Tom Sensenig, now with USFS, was not informed of the paper. "It was quite a surprise to have a cooperative agreement turn into a publication that was essentially kept secret," says Sensenig, who says he disagrees with the conclusions. In addition, the paper did not get the normal review from USFS or BLM, which Sessions and Sensenig say would have removed what they perceive as political overtones. Ann Bartuska, USFS's chief of research, agrees, but she doesn't see any major problems with the paper: "It's a good piece of work that's adding to the discussion."

Donato denies any political agenda and says the authors referred to the bills to highlight the timeliness of the research. There was no intention to avoid reviews, Donato says, but he declines to elaborate on that or why Sensenig wasn't included. "It was a misunderstanding," he says. Donato says he and his co-authors will respond to technical criticisms in the peer-reviewed literature. (Sessions and his colleagues plan to submit a technical comment to *Science*.)

Meanwhile, the dean of OSU's college of forestry, Hal Salwasser, has tried to calm the waters. A first attempt backfired when some students and faculty members interpreted a memo as criticizing Donato and his co-authors. On 26 January, Dean Salwasser wrote another department-wide e-mail in which he praised the authors for having a paper accepted at *Science* and reiterated a commitment to academic freedom. "I profoundly regret the negative debate that recent events have generated," he wrote. He has set up a committee on academic freedom within the college.

Sessions isn't backing off. He says he will press the board of AAAS (*Science*'s publisher) to investigate what he sees as shortcomings in peer review. Donato is hoping to be able to concentrate on his research sometime soon. "This has dominated my waking hours," he says. "It's been really crazy."

–ERIK STOKSTAD

A promised 10-year doubling for NSF, NIST, and energy research would be offset by no growth for NIH and NASA in President George W. Bush's spending request for 2007

A Budget With Big Winners and Losers

PRESIDENT GEORGE W. BUSH'S BUDGET for next year answers a fervent wish by the scientific community for a boost to the physical sciences, more attention to science and math education in the public schools, and a focus on applied energy research. But in trying to balance the costs of two wars and additional tax cuts with a desire to trim spending, the president's budget would also flat-line the National Institutes of Health (NIH) and freeze NASA's spending on earth and space sciences for the next 5 years. That exercise, unveiled this week, could trigger a donnybrook among the various disciplines for their share of the pie as the 2007 budget wends its way through Congress.

Predictably, the community is divided on the merits of the president's latest budget request. "It's a historic moment," says Michael Lubell of the American Physical Society. Not quite, says former NIH director Harold Varmus, head of the Memorial Sloan-Kettering Cancer Center in New York City. Although he's "pleased by" the boost to the physical sciences, "I'm fairly disturbed by what's happening to NIH." The 1989 Nobel laureate says he fears that "NIH is going to be worse off than it was at the beginning of the Bush Administration."

In a lean budget year, says presidential science adviser John Marburger, scientists should be grateful for any increases. The 14% rise at the Department of Energy's (DOE's) Office of Science and the 7.9% boost for the National Science Foundation (NSF), he says, represent "high-priority areas … that are most likely to generate the sort of results that will create technologies to improve U.S. competitiveness." The increases are part of what the White House is calling the American Competitiveness Initiative, a bundle of proposals that includes the promise of a 10-year budget doubling for NSF, DOE's Office of Science, and core research at the National Institute of Standards and Technology (NIST).

It also represents a vote of confidence in those agencies to deliver the goods. In contrast, Marburger says, NIH's no-growth $28.6 billion budget reflects "an agency in transition" that is struggling to digest a recent 5-year doubling of its budget. And NASA, which would receive a 1.5% increase for science in 2007 and less down the road, "is grappling with a lot of issues."

From the top

The competitiveness initiative is the product of a bevy of recent reports from both internal and outside advisory groups, and a lobbying effort by science and business groups that gathered momentum last fall (*Science*, 21 October 2005, p. 423). "Everything came together, and the president realized that there were opportunities," says Marburger.

Researchers, especially those in the physical sciences, hope to reap the rewards. In nuclear physics, for example, proposed $87 million boost would allow DOE to keep open both of its large nuclear physics labs instead of having to choose between Brookhaven National Laboratory (BNL) in Upton, New York, and Thomas Jefferson National Accelerator Facility (JLab) in Newport News, Virginia. "Pinch me," says Samuel Aronson, BNL's associate director for high-energy and nuclear physics. BNL also would receive $45 million from the DOE basic energy sciences program to upgrade its synchrotron light source. The proposed increase may also allow JLab officials to stave off planned involuntary layoffs caused by a tight budget this year. Within an 8% increase for high-energy physics is $10 million to design a new neutrino experiment at Fermi National Accelerator Laboratory in Batavia, Illinois.

NSF-funded researchers are also likely to cash in if Congress goes along with the competitiveness initiative. The foundation's proposed boost includes an estimated 500 new research grants across all disciplines and a $5000 increase in the average annual grant size, to $148,000. NSF received a green light to start two projects at the top of its wish list, the Alaska Region Research Vessel and the Ocean Observatories Initiative. Its polar research programs would get a $48 million jump, to $370 million, including several new projects for scientists participating in the International Polar Year program. And NSF has requested a $50 million down payment on

CREDIT: CHRIS MADDALONI

On point. Science adviser John Marburger says the president's 2007 budget bolsters science.

a peta-scale computing network that can handle the increasingly large databases that scientists collect and analyze.

The Department of Homeland Security would like $18 million for research on nuclear detection and forensics. "We want to step up basic research efforts to extend the range and accuracy of nuclear detection," says Vayl Oxford, head of the department's new Domestic Nuclear Detection Office. One goal is a detection system capable of sniffing out radiological materials on cargo ships at sea. NSF is hoping for $20 million for a new competitive research program to develop better sensors to detect improvised explosive devices, a deadly weapon of choice in Iraq.

DOE's work in advanced computing would get a 37% ($84 million) increase, which managers say will allow them to install a raft of much-needed new processors. These would help alleviate the crunch at the National Energy Research Scientific Computing Center at Lawrence Berkeley National Laboratory in California. Director Horst Simon says he is "really satisfied," because his facility is currently "oversubscribed by a factor of 3 or 4."

Work in basic energy and materials science at DOE received special attention in the budget, including the Spallation Neutron Source at Oak Ridge National Laboratory in Tennessee expected to open in June and the Linac Coherent Light Source at the Stanford Linear Accelerator Center in California. The money will help "projects that couldn't be fully funded in '06," says physicist Gabrielle Long of Argonne National Laboratory in Illinois. More than $300 million in new spending on applied energy research—part of the president's pledge to curb U.S. "addiction" to oil—would target photovoltaics, advanced nuclear fuel processing, the conversion of cellulose to ethanol, and wind power. The Administration has resisted new funding for many of these same programs in previous budgets.

Areas with a similarly promising economic upside held sway at NIST. Its Center for Neutron Research is slated to receive $12 million for new instrumentation and $10 million to beef up its programs. "The mood is ecstatic," says Patrick Gallagher, who heads neutron science research at NIST in Gaithersburg, Maryland. About half of the $20 million increase for nanotechnology would fund a center on transitioning technologies from initial discovery to production. Hydrogen economy research is also slated for a $10 million increase. "We've had very flat budgets for a long time," says Eric Steel, who oversees the program office at NIST in Gaithersburg.

The Administration says NIST's core research programs would jump by 24% under the request, although the total—$535 million—is actually less than current funding levels. The reason, says Marburger, is that the budget doesn't include funds to continue projects the agency did not request but Congress bankrolled to the tune of some $135 million in 2006. Many of these so-called congressional earmarks have little to do with NIST's mission, he adds.

The White House initiative also targets math and science education, under the leadership of the Department of Education. The budget includes $326 million—51% more—than last year for the department's activities in this area, including programs to strengthen research-based math instruction at elementary schools and to help math-deficient middle school students take and pass algebra. The president is asking Congress to add $90 million to a $32 million program to increase the number of students passing Advanced Placement tests in math and science by preparing more teachers and rewarding students. The Administration also hopes that local schools will recruit 30,000 working scientists and engineers as part-time teachers and is offering school districts $25 million to implement such programs.

With science education a renewed White House priority, NSF's $800 million education directorate would receive a 2.5% increase. The biggest winners are three undergraduate and graduate programs intended to increase the number of minorities going into science and engineering. They would get a 24% boost, to $85 million. "We've seen a linear curve with a low slope for a long time," says NSF Director Arden Bement. "And we've been patient. But now we want to be impatient."

2007 U.S. Budget Highlights (In $ Millions)

	2006	2007 request	Annual % change
NIH	28,587	28,587	0.0% ↔
NSF	5,579	6,021	7.9% ↑
Research	4,331	4,665	7.7% ↑
Education	796	816	2.5% ↑
NASA	16,623	16,792	1.0% ↑
Science	5,254	5,330	1.5% ↑
Exploration	3,050	3,978	30% ↑
Department of Defense basic research	1,470	1,422	–3.3% ↓
DARPA basic research	133	151	13% ↑
Department of Energy Office of Science	3,596	4,102	14% ↑
High-energy physics	721	775	8% ↑
Basic energy sciences	1,135	1,421	25% ↑
Nuclear physics	367	454	24% ↑
Advanced (nuclear) fuel cycle	79	243	208% ↑
Department of Commerce			
NOAA oceanic and atmospheric research	370	338	–9% ↓
NIST science and technology research	568	535	–5.8% ↓
Environmental Protection Agency R&D	563	528	–6.2% ↓
U.S. Geological Survey	971	945	–2.7% ↓
USDA's competitive research	181	248	37% ↑
Multiagency initiatives			
Networking and IT	3,017	3,089	2.4% ↑
Nanotechnology	1,299	1,275	–2.0% ↓
Climate change science	1,713	1,717	0.2% ↑
Total Security	**73,730**	**75,742**	**3.0%** ↑
Total Civilian	**60,051**	**61,462**	**2.3%** ↑
Total R&D*	**133,781**	**137,204**	**2.6%** ↑

* Includes agencies not listed.

SOURCE: WHITE HOUSE OFFICE OF MANAGEMENT AND BUDGET AND INDIVIDUAL AGENCIES

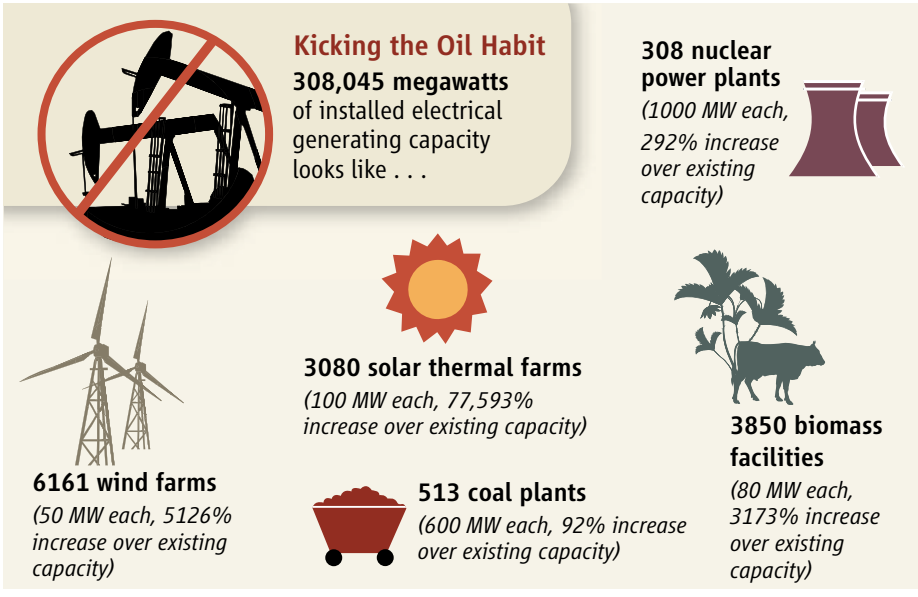
Lagging behind

The proposed $20 million increase in the NSF education directorate would reverse double-digit declines proposed by the Bush Administration in its previous two budgets, which Congress partially reversed. But the $816 million requested is still $27 million less than the directorate received in 2005. The biggest loser is NSF's shrinking Math and Science Partnerships program linking university professors with local school districts. First proposed in 2002 as a $200 million initiative, the program would limp along at $46 million for existing projects, down $17 million from this year, with no new competitions.

The consequences of setting priorities is a common theme among agencies that took a hit in the president's 2007 budget. "We're not in a position to do as much as many of us would like," says Michael Leavitt, secretary

of the Department of Health and Human Services, which oversees NIH. Asked why biomedicine was not included among the science agencies funded under the competitiveness initiative, NIH Director Elias Zerhouni replied: "I don't think biomedicine is necessarily less urgent, … but you have to make choices that are not necessarily going to make everybody happy."

whose current $4 billion budget would drop by $279 million, will be hard-pressed to achieve the goals laid down last year by the commission. And funding for the Oceanic and Atmospheric Research program—which includes the state-based research and education Sea Grant network—has been sinking steadily, from $404 million in 2005 to a proposed $338 million in 2007. The commission "proposed a lot



Oil alternatives. In his State of the Union speech, President George W. Bush set out a goal to find alternatives for 75% of the oil that the United States expects to be importing from the Middle East by 2025. According to the Energy Information Administration, the total then will be 5.99 million barrels of oil per day from the Persian Gulf, up from 3.7 million barrels in 2005. So the country must replace 4.49 million barrels a day, or 1.64 billion barrels a year. Here's what it would take for each power source (*above*) to generate an equivalent amount of electricity. (Considerably more would be needed to convert the electricity into a transportation fuel.)

Indeed, there is little in NIH's 2007 budget to cheer biomedical researchers. Biodefense would garner a significant increase—some $110 million for a new biodefense fund to help universities and companies commercialize countermeasures. And Zerhouni's road-map initiative to fund programs that cut across NIH would receive a $113 million boost, to $443 million. NIH would also spend $49 million more to expand an initiative on genes, environment, and health, and $15 million for a new bridge award for young investigators. Most of NIH's 27 institutes and centers would get a slight cut under the president's plan. As a result, success rates on grants—an investigator's odds of winning funding for a grant proposal—would remain at 19% in 2007, down from 22% in 2005.

The bad news for scientists extends beyond NIH. The science and technology account at the Environmental Protection Agency would go down by 6.7%. For ocean science, Bush's budget won't go very far in achieving the goals of the congressionally mandated U.S. Commission on Ocean Policy. The National Oceanic and Atmospheric Administration,

of great new programs," says Beth Lowell of Oceana, a Washington, D.C.–based nonprofit. "With the current budget cuts, I am unsure [any of] that will happen."

The prospects for NASA-funded scientists are among the bleakest in the federal government. NASA Administrator Michael Griffin did not sound happy as he described a 1% increase for his agency, although he insisted the real figure is 3.2% if a $350 million supplement for Hurricane Katrina recovery in 2006 is not included. But even that rise is a far cry from the 8.8% increase he sought to deal with rising space shuttle and space science costs while he tries to get an expensive human exploration effort off the ground (*Science*, 6 January, p. 28). Faced with a flat budget for the next 5 years, he said, "we took a couple of billion [dollars] out of science" as well as money from exploration.

Those couple of billion dollars in cuts would be spread out over the next 5 years. So although NASA's science program would increase slightly in 2007, the impact on specific missions will be dramatic immediately. The agency is dropping a host of missions on hold, including a space interferometry telescope, a probe designed to search for Earth-like planets, and a spacecraft to measure global precipitation, says NASA science chief Mary Cleave. Spending on Mars robotic missions through 2011 would be reduced by nearly half of what was envisioned just last year; plans for a Mars sample return and a telecommunications orbiter will be abandoned.

Projects running over budget are in particular danger. The new request, for example, provides no funding for the Stratospheric Observatory for Infrared Astronomy, which is nearly ready to fly but has encountered cost and technical problems. In addition, work on the asteroid mission called Dawn is on hold pending review.

By contrast, NASA spending on exploration systems would jump from $3 billion to $4 billion in 2007. The small piece of that budget devoted to human research, however, would shrink by 56% to $275 million, given NASA's decision to minimize scientific work on the international space station. Griffin insists that science at NASA has increased significantly during the past decade, but "we cannot afford such growth" in coming years.

The raid on science is already meeting strong resistance. Hours before Griffin met with reporters, House Science Committee Chair Sherwood Boehlert (R–NY) said he was "greatly concerned" with the "sharply reduced growth" in space and earth sciences in the NASA request. And Wesley Huntress, a former NASA science chief and president of the Pasadena, California–based Planetary Society, criticized the request for "using money intended for science programs to fund continued operation of the shuttle, … a program scheduled for termination."

In the end, those who have done well are singing the White House's praises, while those who didn't are questioning its judgment. "This has been a remarkable year for advocacy," Bement said to an audience of science lobbyists who applauded his festive presentation. "There has been a lot of rhetoric about doubling, and now the president is behind it."

For the Federation of American Societies for Experimental Biology in Washington, D.C., however, the NIH budget is a reason for "disappointment and outrage." Says Patrick White of the 62-member Association of American Universities, "What they have done for physical science is incredible. What's unfortunate is that they're allowing NIH to wither on the vine." A coalition of advocacy groups will be pushing Congress to give NIH a 5% increase.

–ELI KINTISCH AND JEFFREY MERVIS

With reporting by Yudhijit Bhattacharjee, Adrian Cho, Jocelyn Kaiser, Andrew Lawler, Elizabeth Pennisi, and Robert F. Service.

CREDIT: K. BUCKHEIT/*SCIENCE*; SOURCE: ENERGY INFORMATION ADMINISTRATION

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ANIL KAKODKAR INTERVIEW

Breaking Up (a Nuclear Program) Is Hard to Do

India nuclear chief Anil Kakodkar has no apologies for staking out a tough line on implementation of a landmark India-U.S. nuclear pact—even if that sinks the deal

NEW DELHI—Anil Kakodkar is a legendary figure in India's rise to nuclear statehood. Now pressure is building on the self-described technocrat to prove his diplomatic mettle as well. A historic nuclear agreement between India and the United States is riding on India's plan to segregate its nuclear establishment into civilian and military components (*Science*, 20 January, p. 318). As chair of India's Atomic Energy Commission in Mumbai and secretary of the Department of Atomic Energy, an agency with 65,000 staff and a $1.2 billion budget, Kakodkar has been asked to draw the civil-military line.

The stakes are high. The India-U.S. agreement, signed on 18 July 2005, would end a 30-year embargo on nuclear trade with India stemming from its refusal to sign the Nuclear Nonproliferation Treaty. As part of the deal, India has committed to designating which of its nuclear facilities are civilian and can be placed under international monitoring. Those labeled military would be neither under safeguards nor eligible to receive imported nuclear technologies or fuel. Before the agreement can go ahead, the U.S. Congress must amend laws; congressional action will hinge on acceptance of India's separation plan.

In negotiations since December, India has taken a hard line, tagging all nuclear R&D facilities, including its fast-breeder reactors, as military. In a sign of how fraught the talks have become, Kakodkar acknowledges that India and the United States may fail to reach an accord: "India's nuclear program will go on with or without the cooperation," he says.

How much India compromises will depend on Kakodkar, a mild-mannered but tough negotiator who assiduously avoids the spotlight. Kakodkar, 63, trained as a mechanical engineer before joining India's premier nuclear weapons lab, the Bhabha Atomic Research Centre (BARC), in Mumbai in 1963. He says he leads a spartan life, having spent 18 hours a day over the past 4 decades "living atomic energy." He takes pride in having overseen the design of reactors, including the 100-megawatt Dhruva research reactor, which produces plutonium for the country's arsenal, and future reactors unique to India that will run on thorium.

Kakodkar spoke last week with *Science* about everything from the separation plan to India's refusal to contribute real-time seismic data to an evolving Indian Ocean tsunami warning system (*Science*, 9 December 2005, p. 1604). The following transcript was edited for clarity.

Q: What is happening with the Indo-U.S. nuclear deal? Is the separation plan the sticking point?
The determination of what is in the civilian domain … is an Indian determination, and we think that we have done a very objective job. That is what we understand, right or wrong.

Mild-mannered but hard-nosed. The fate of a landmark India-U.S. nuclear agreement appears to rest on Anil Kakodkar's judgment of how much of India's nuclear establishment can be placed under the watchful eyes of international inspectors.

Q: You are not averse to the idea of separation?
No, not at all. But at the same time we cannot allow our strategic interest to be determined by others. We have never had any problem in getting reactors or fuel from outside and putting them under safeguard. We have done that in the past, so we can do that again. We will put some of the indigenously built reactors also under safeguard. But then I have to maintain some proportion outside safeguards, and that proportion has to be based on a good strategic calculation. Now, if somebody says, 'No no, you should put this also under safeguard,' then there is a problem. This is what is under discussion.

Q: If you need plutonium from a military reactor to fuel the fast-breeder reactors, does this linkage mean that the breeders cannot be monitored?
That is absolutely the point.

Q: So categorically the breeders will not go under safeguards?
No way, because it hurts our strategic interest.

Q: The strategic interest of security or strategic interest of energy security?
Both. It hurts both because it is linked through the fuel cycle. Putting the Fast Breeder Program on the civilian list would amount to getting shackled, and India certainly cannot compromise one security for the other.

Q: Is your strategic need for plutonium not met by CIRUS [a research reactor that India acquired from Canada in 1956] and Dhruva? Do you need additional capacity from civilian reactors?
Yes, very clearly. Not from civilian reactors, but from power reactors.

Q: But then where is a compromise likely, with the United States insisting that you put the breeders and part of your power reactors under safeguards?
We have to discuss that logic. In fact, it goes beyond the July 18 statement. It amounts to changing the goalposts.

Q: What amounts to changing the goalposts? Asking for the breeders under safeguards?
Asking for a specific thing to be put under safeguards. That amounts to changing the goalposts.

Q: If the political leadership demands it, would you be willing to accept changing the goalposts?
Where is the question of my willingness? I am a

technocrat, and I will point out all the ramifications. It is as simple as that.

Q: So what will happen to CIRUS? Why isn't it on the civilian list?
With CIRUS we have gone through a whole refurbishing, everything has been changed.

Q: Anything original left, other than its name?
Well, I think there is some concrete somewhere. But, jokes apart, it has undergone substantive refurbishing. The second point is that CIRUS is located in BARC, which is a strategic facility. We maintain that CIRUS has always been in use for peaceful purposes. At the same time, you cannot put CIRUS under safeguards simply because it is inside BARC.

Q: If the nuclear pact is realized, do you foresee joint reactor development with the U.S.?
Let us not speculate too much; it would be daydreaming. India will build its own innovative reactors—that much I can tell you.

Q: What is your view on sharing seismic data?
The waveforms of earthquakes contain a lot of information, and some locations could be sensitive. What is, after all, seismic monitoring? You get information on a disturbance in the earth, measured at some location. This measurement contains both information related to the source of the disturbance and information related to the path through which this disturbance travels up to the measuring point. You have to have policies that if the earthquake is more than some magnitude, data are released. Many countries do this. So we should have our own policy. That does not mean that we are against stopping everything, but it cannot be a free-for-all.

Q: You are willing to consider a change in the policy?
There is no policy. The point is, let there be some policy, and things should be done according to that policy.

–PALLAVA BAGLA

Fish sense. Gars (*left*) can help zebrafish researchers understand the evolution of development.

Fishing for Common Ground

Biomedical and basic researchers who study fish face challenges in combining forces to understand development and evolution

When a traditional fish biologist meets a zebrafish researcher, things don't always go swimmingly. The former cares about how fish behave in their watery world and how evolution has shaped piscine diversity. The geneticists and developmental biologists who study zebrafish generally have a biomedical bent. "We tend to think of it as a wet mouse with a transparent embryo," says John Postlethwait, a zebrafish expert at the University of Oregon, Eugene. "We tend to have very little appreciation for our organisms as fish."

But as a recent meeting in Orlando* made clear, the two camps are increasingly finding common water. For more than a decade, the zebrafish has been one of several crucial model organisms for developmental biology (*Science*, 30 August 2002, p. 1484). Its genome is already sequenced, and there are many molecular techniques to help researchers glean this species' biological secrets, and in turn, better understand all vertebrates, including humans. Those tools are now proving seductive to biologists trying to understand the genetic basis of all fish evolution. At the same time, the zebrafish community is beginning to realize that examining different fish species can help them understand the functions of the genes they find.

Take Mark Cooper, a developmental biologist at the University of Washington, Seattle. "I need a roundtable session in Orlando set up to build bridges between the two fish factions. Cooper has studied how the first embryonic cells begin to establish a body axis in the zebrafish, and he is retracing the history of this key developmental step, called gastrulation, by looking at the process in other fish. "How about a gar?" Richard Mayden, a systematist at Saint Louis University in Missouri, piped up. "I've got a contact."

Cooper was ecstatic. He's been working through branches of the fish family tree looking at similarities and differences in gastrulation but has not been able to reach the lower, oldest limbs. Gar, long, narrow fish with sharp teeth whose fossil history traces back to the Permian, are a perfect complement to other species he's been examining. The ability to look at a developmental process in multiple species "opens up a huge window into the past," says Cooper.

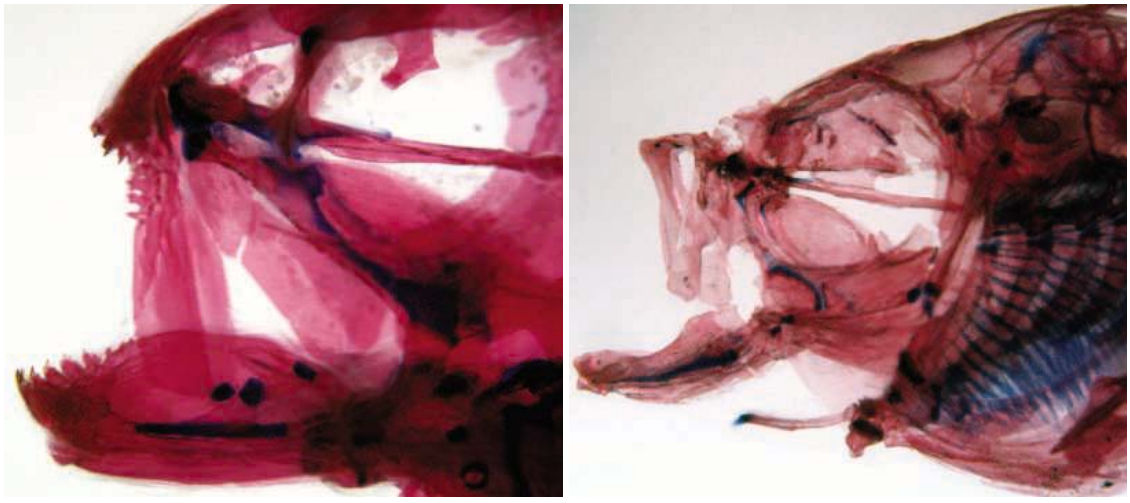
Smoothing rough waters

Traditional fish biologists pride themselves on a great respect for their organisms, focusing on a fish's natural history and its phylogenetics. Yet they sometimes have a fear and loathing of molecular endeavors, says Jacqueline Webb, a fish biologist at Villanova University in Pennsylvania. Comparative biologists also concentrate on adult morphologies and behaviors, and their raw material is physical specimens, often from collections in natural history museums. Biologists working on zebrafish, on the other hand, tend to focus on early development and prefer to compile their data as digital images. Given such differences, until recently, "neither community has felt there is much to discuss," says Paula Mabee, an evolutionary developmental biologist at the University of South Dakota, Vermillion.

Efforts such as the Cypriniform Tree of Life Project, which Mabee helps coordinate,

* The annual meeting of the Society for Integrative and Comparative Biology was held in Orlando, Florida, 4 to 8 January.

CREDIT: SUSAN MIDDLETON/CALIFORNIA ACADEMY OF SCIENCES



Toothless. Comparative studies of the Mexican tetra (*left*) and the zebrafish (*right*) are revealing how fish gained and lost teeth through time.

are beginning to stimulate more dialogue. This National Science Foundation–funded venture should sort out the evolutionary history of cypriniforms, the diverse group of fishes that includes the zebrafish. Together with colleagues from China, Japan, and other countries, Mayden and Mabee are gathering morphological data and DNA from museum specimens and collecting species from far-flung places to determine the genetic relationships between 1000 of the 3200 known cypriniforms. "We are not just building a tree. We are working with the zebrafish people to learn from them and for them to pick up things from us," notes project director Mayden.

In another effort at détente, Mabee and her colleagues are trying to build a common vocabulary among fish biologists in order to make interactions easier. Working with curators of the online Zebrafish Information Network (ZFIN), they are coming up with an "ontology," a standardized list of terms for describing traits, that will make possible one-click shopping at both the Cypriniform Tree of Life database and ZFIN. "By overlapping these two sets of data, we can get candidate genes for evolutionary phenotypes," says Mabee. Her colleagues are excited at the prospect. "The sharing of database and informatics is a delightful example of the cooperation between the two fields that should be hugely helpful," says Charles Kimmel, a zebrafish biologist at the University of Oregon, Eugene.

One person who may soon benefit from all this new cooperation is Thomas Schilling, a veteran zebrafish developmental biologist at the University of California, Irvine. Schilling wants to broaden his research on skull and facial developmental to understand its evolution in fish, so he needs to identify the right species for such comparative work and find ones he can raise in the lab. "Yet, I'm not really aware of what other [fish] are easy to work with," he complains.

The systematists participating in the Cypriniform Tree of Life Project are coming to his rescue. This project boasts of 30 cypriniform species in labs across the world, with more breeding programs in the works, some by high school students. The researchers also know aquarium dealers, hobbyists, and fish importers who may be ready sources of fish. "Just give me your wish list, and I will come up with the material," Mayden says.

Traditional fish biologists are in turn getting help from the zebrafish community. Amy McCune, an evolutionary biologist at Cornell University, is combing the zebrafish databases for mutants with missing or dysfunctional swim bladders. These organs are critical to most fish, but a few species in more than a dozen fish families have done away with the swim bladder, and McCune wants to know how. Once she identifies genes that control the development of the zebrafish bladder, she can turn to the bladderless species and examine what happened to those genes in each species.

In contrast, David Stock, an evolutionary biologist at the University of Colorado, Boulder, is looking into something most fish have but zebrafish don't: mouth teeth. His work in zebrafish implicated two genes, *Dlx* and a *Dlx* regulator, fibroblast growth factor (FGF), in depriving the zebrafish of mouth teeth. When he inhibited FGF in the Mexican tetra, a close, toothed relative, its teeth also failed to form, Stock reported at the meeting. The finding suggests a possible mechanism by which teeth were repeatedly lost and gained over time in fish, he says.

David Parichy, an integrative biologist at the University of Washington, Seattle, straddles both worlds in his work on the diversity of color patterns in fish. Cypriniforms can have any number of stripes or bars, and Parichy is curious why and how this variety evolved. Using zebrafish, he and his colleagues have found that two groups of pigment precursor cells work together to form that animal's stripes—one group forms early in development and the other group develops later.

Stripe potential. Differences in how two groups of pigment precursor cells interact lead to stripes in the zebrafish (*top*) but no stripes in the closely related pearl danio (*bottom*).

A close zebrafish relative, the pearl danio, has no stripes, however. In Orlando, Parichy reported that when his team eliminates the earlier-forming precursor cells in pearl danio, the fish develops stripes. They are not quite as dark as in the zebrafish, but they are clearly visible. "The work is probably the best example of a zebrafish-focused study which explains the diversity in a related species at the developmental genetic level," says Stock.

Work like Stock's, McCune's, and Parichy's may not be mainstream yet, but it's no longer off in an isolated eddy. "What you are seeing are both sides adapting the approaches of the other," says Cooper.

–ELIZABETH PENNISI

CREDITS (TOP TO BOTTOM): DAVID W. STOCK; DAVID M PARICHY

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EVOLUTION

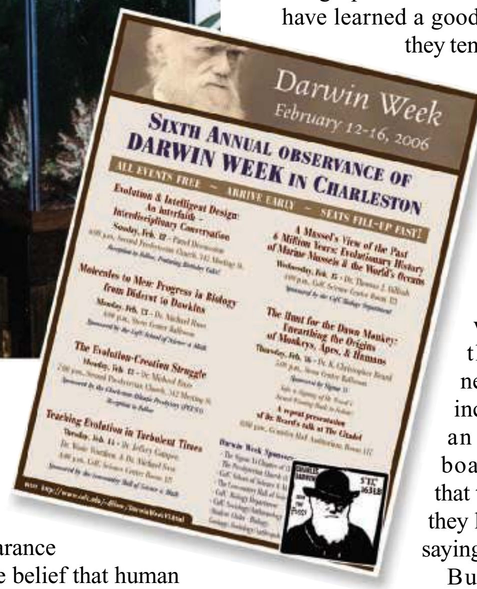
Darwin's Place on Campus Is Secure—But Not Supreme

Professors at many U.S. universities say their students are learning about evolution without abandoning their belief in some form of creationism

During a visit to Stanford University in 1994, Cornell University biologist Will Provine bet geneticist Marcus Feldman that there were "a bunch of creationists" among undergraduates at the prestigious California school. He says Feldman scoffed. But when Provine asked Feldman's biology students "how many of you believe humans came to be in the last 10,000 years?" a sizable number raised their hands.

Provine says there's no evidence that much has changed since then. The debate over evolution has heated up in recent years, with creationists and proponents of intelligent design (ID) clamoring for a place in the curricula of public schools around the country (see sidebar, p. 770). Ironically, this is occurring in the face of an expanding application of evolutionary theory throughout the sciences. Yet polls indicate that the proportion of Americans whose beliefs lie somewhere in the creationism spectrum has held steady for decades.

Interviews with two dozen professors suggest that the same firmness of conviction can be found on many U.S. campuses. "Students may become more accepting of evolution, but they don't throw out creationism," says biology professor Randy Moore of the University of Minnesota, Twin Cities.



Feting the founder. Celebrating Darwin Week in South Carolina means posters at the College of Charleston and a change of costume for biologist Jerry Waldvogel of Clemson College.

CREDITS: COURTESY OF JERRY WALDVOGEL; (INSET) COLLEGE OF CHARLESTON

Hard-core beliefs

For decades, polls have indicated that close to half of the U.S. adult population is skeptical of the basic tenets of Darwinian evolution. Although more educated people are more likely to endorse evolution, a college degree is no guarantee that the graduate agrees with Darwin.

Provine himself has been surveying his Cornell students since 1986, when he started teaching an evolution course for nonbiology majors. He says that for many years, about 70% of students held views somewhere along the creationist spectrum, from biblical literalism about the sudden appearance of Adam and Eve to the belief that human existence could not have come about without divine intervention. The percentage holding those views declined after agriculture and business students were no longer required to take the course, he says, but not enough to make them stand out from the general population. "Human evolution is a flash point; that's where the rubber meets the road," says biologist James Colbert of Iowa State University, Ames. "It's very common to see students who simply can't believe humans evolved from apes."

For the past 3 years, Colbert has surveyed students in his introductory biology class, asking them if they believe God created humans within the past 10,000 years. Last fall, 32% of their 250-member class said they did, Colbert says he finds this percentage particularly unsettling "when one considers that these students are academically among the upper half of high school graduates, and they are students choosing to major in a life science"—often to become doctors or veterinarians.

For the past 5 years, Moore has done the same surveys in his giant introductory biology class at Minnesota. He says only a little more than half of his students say they were taught anything about evolution in high school; of those, about half say creationism was discussed. That jibes with figures from teacher surveys in both 1994 and 2004, in which one-quarter report that they talk about creationism in their biology classes.

Moore says students don't necessarily know how to define ID, which asserts that there must be a "designer" because life forms are too complex to have arisen solely from the process of random mutation and natural selection. But when Moore presents them with a range of beliefs, 15% to 20% side with the ID movement. And "virtually none" has changed his or her mind by the end of the semester, he notes. Colbert agrees that although postcourse surveys show students have learned a good deal about evolution, they tend to stick to their views on God's role in creating humans.

Plant biologist Massimo Pigliucci of Stony Brook University in New York says he encountered "all sorts of interesting reactions" when he taught at the University of Tennessee, Knoxville. They included notes posted on an Internet discussion board warning students that they would go to hell if they listened to what he was saying about evolution.

But teachers say they rarely have in-class clashes with such students. Rather, says biologist Robert Dillon of the College of Charleston in South Carolina, students will come by "several times a semester" to express their concern that "if there was no Adam, that means Christ died in vain for our sins. We'll have a theological discussion," he says.

The discussions aren't limited to biology courses. Geologist Robert C. Thomas of the University of Montana–Western in Dillon says he is encountering a growing number of students "who do not understand or believe in the most basic concepts of geologic time and evolution," and that they have become "far more vocal and in some cases disruptive" in

Is ID on the Way Out?

Last month, a teacher in a rural southern California high school began a monthlong course on the "Philosophy of Design," exploring issues such as "why is intelligent design [ID] gaining momentum?" In response, 11 parents, with help from Americans United for the Separation of Church and State, sued the El Tejon Unified School District on 10 January. Fresh from a decisive December win over proponents of ID in Dover, Pennsylvania, evolution's defenders geared up for another court battle.

But they didn't get one. Facing projected legal costs of $100,000, the school board agreed to a settlement, ending the course early and promising not to teach any course that "promotes or endorses creationism, creation science, or intelligent design."

For some observers, the board's swift capitulation was further proof that the ID movement has crested. Although the specifics of the cases were different, "the very decisive win in Dover meant [the California board] knew they had no chance of winning this," says philosopher of science Robert Pennock of Michigan State University, East Lansing, an expert witness in Dover. "ID is on its way out," agrees evolutionary biologist Joel Cracraft of the American Museum of Natural History in New York City, who has been active in defending evolution. "[Creationists] will be avoiding that term."

Indeed, the leaders of the ID movement prefer a more subtle approach to undermine the teaching of evolution: Urge schools to teach the "controversy" over evolution. "We oppose mandating the teaching of ID," says John West of the Discovery Institute in Seattle, Washington, the leading promoter of ID. "We opposed that [El Tejon] class," which was laden with young-Earth creationism as well as ID; the institute also opposed the Dover policy. Their latest video for school districts, entitled "How to Teach the Controversy Legally," does not mention ID.

Such language is echoed in the draft Kansas Science Standards (*Science*, 4 November 2005, p. 754), which call on teachers to teach the evidence "for and against" evolution, as well as in the warning labels put on textbooks in Cobb County, Georgia (*Science*, 21 January 2005, p. 334). Much of this year's crop of antievolution legislation follows suit. A Michigan bill, for example, proposes that students "critically evaluate scientific theories including, but not limited to, the theories of global warming and evolution."

Given these shifting tactics, the battle over teaching evolution "isn't over," says Alan Leshner, CEO of the American Association for the Advancement of Science, which publishes *Science*. "These people are well-financed and ideologues in the true sense, and they are not giving this up."

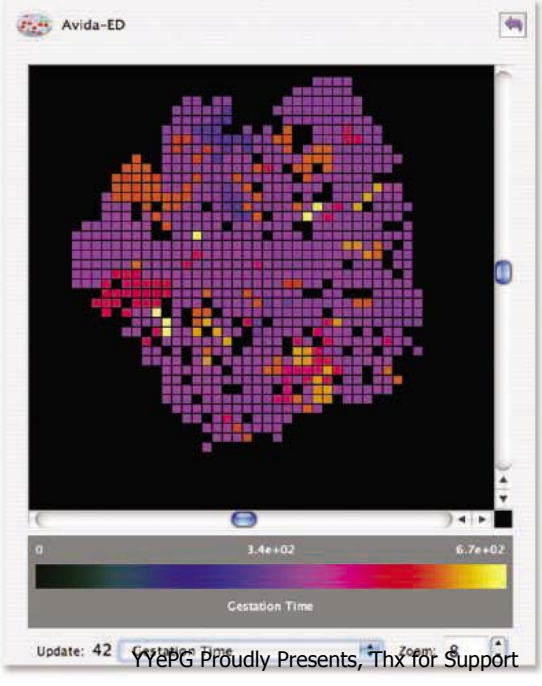
–ELIZABETH CULOTTA

Poor design? The El Tejon class drew protests before it was canceled.

class. "I think the earth sciences are on the front lines of this battle," says geologist Joseph Meert of the University of Florida, Gainesville. "If you have an old earth, evolution has a chance to happen."

Last fall, the Geological Sciences of America (GSA) meeting in Salt Lake City, Utah, featured a panel on young-Earth creationists. GSA sees the movement as "a serious attack on legitimate science, not just evolution," says geologist Edward Crisp of West Virginia University, Parkersburg. He says that although most students will accept the validity of the scientific method, more than half fall away "when you throw man into the mix and ask about a common ancestry with great apes."

Crisp surveyed students in several introductory biology classes this winter and found that 25% of 206 students believed in a young Earth. The postcourse surveys of 115 students showed that 17% retained that belief. Asked after the course if they accepted biologic evolution as a "fact," one-third expressed doubts. That's not a big drop from the 42% in the precourse survey who had doubts. In answer to a separate question, about half said creationism should get equal time with evolution in public schools.



Virtual petri dish. Avida-ED allows students to track mutations, proliferation, metabolic rates, and other bacterial characteristics on their computers.

Why the resistance to change? "Sometimes students want to take science courses so they can get better in their arguments with scientists," explains Crisp. He adds that although most of his students won't become scientists, they may still be in a position to influence the young. "Over 50% of my students are majoring in elementary education," he notes.

Teaching in the city that hosted the infamous 1925 Scopes Trial, invertebrate paleontologist Kurt Wise of Bryan College, a Christian school in Dayton, Tennessee, says other scientists have an exaggerated fear of fundamentalists like himself. (Wise claims to be the first "young age" creationist with a doctorate in paleontology, earned in 1989 from Harvard University.) After all, he notes, "if you're working for an oil company, it doesn't matter if you think the oil is only 500 years old."

But Wise's is distinctly a minority view. Most geologists agree with Meert when he

CREDITS (TOP TO BOTTOM): NIKLAS LARSSON/AP PHOTO; R. T. PENNOCK/MICHIGAN STATE UNIVERSITY

says that "it's time to stop pussyfooting around. … Young-Earth creationism and the ID movement are challenging the foundations of not just biology but also geology, physics, chemistry, astronomy, and anthropology."

Darwin days

Public controversies over Darwinism have inspired college presidents to defend science and professors to sign petitions. They've also inspired courses to explore the evolution debate. University of Kansas religion professor Paul Mirecki made national headlines when he announced a course that would label ID as "religious mythology." Mirecki was subsequently beaten up by thugs and excoriated when some fundamentalist-bashing—and Catholic-bashing—e-mails he had written became public. He also stepped down as department chair, although university officials say they still hope to offer such a course.

But for all the media coverage of the controversy, few academics are proposing new approaches to teaching evolution in biology or geology class. "There are fewer people than I would have thought trying to reach out" to skeptical students, says physicist Lawrence Krauss of Case Western Reserve University in Cleveland, Ohio, who has been active in the public debate over teaching creationism and ID in public schools. Brown University biologist Kenneth Miller, who has been publicly confronting creationists for years, says he's not aware of any attempts to recast courses in light of the current controversy. But he says evolutionary concepts are dispersing in other ways, in emerging fields such as rational drug design, comparative genomics, and computational biology.

Technology is also providing new teaching opportunities. At Michigan State University (MSU) in East Lansing, scientists are developing a computer program to bring students face to face with evolution. With a grant from the National Science Foundation, a group is adapting a research platform called Avida to enable undergraduates to watch digital organisms called Avideans develop complex functions through replication, mutation, and natural selection.

"The thing we've seen anecdotally is it lets students see that evolution works as advertised," says MSU philosophy professor Robert Pennock. It's "a good way to teach students about the nature of science," says plant biologist Diane Ebert-May, who notes that Avida-ED (as it's called) is also "your best counterattack to ID, which is not science."

Indeed, a much larger reality than the creationism debate is the spread of evolutionary thinking throughout the sciences, including social and behavioral science. Evolutionary biologist David Sloan Wilson of Binghamton University in New York is one scientist who has seized on this phenomenon to generate a program that introduces evolutionary theory to every corner of the university. In 2003, Wilson created a course for nonbiology majors on "evolution and human behavior." His approach was to face moral and political objections to the theory head-on and have students apply evolutionary theory to a wide variety of behaviors, from drug abuse to yawning.

The course, now called "Evolution for Everyone," has spawned a campuswide Evolutionary Studies Program (bingweb.binghamton.edu/~evos) allowing core faculty members to offer courses in virtually any discipline taught from an evolutionary perspective. Outside lecturers are also regularly invited to give public symposia on subjects such as Darwinian medicine or "the deep structure of the arts." Wilson says his surveys show that students are absorbing the basic message regardless of their political or religious orientation. Once

students see evolution not as a dogma but rather as "a powerful way to understand the world," he says, they've "basically been immunized to intelligent design."

Another approach is being developed at the University of Georgia, where evolutionary geneticist Wyatt Anderson, ecologist Patty Gowaty, and others have established a Center for the Study of Evolution. The center will feature speakers from a variety of disciplines, a certificate program, and outreach to public schools. "It's not as evangelical" as Wilson's program, says Gowaty. "We just want the quality of discussion to be better." Anderson hopes the center will also "be a voice for the science of biological evolution" at the state level.

Evolution is also being spread around at the University of Alabama, where faculty members have organized a lecture series called ALLELE, for Alabama Lectures on Life's Evolution. Psychologist David Boles says he got the idea from polls showing that 45% of Americans—and 56% of Alabamans—believe God created humans within the past 10,000 years. Representatives from the education and philosophy departments, as well as various branches of science, design events suited to their fields, and members of the public, especially schoolteachers, are welcome. Geologist Fred Andrus says "we've been very pleasantly surprised at the turnout."

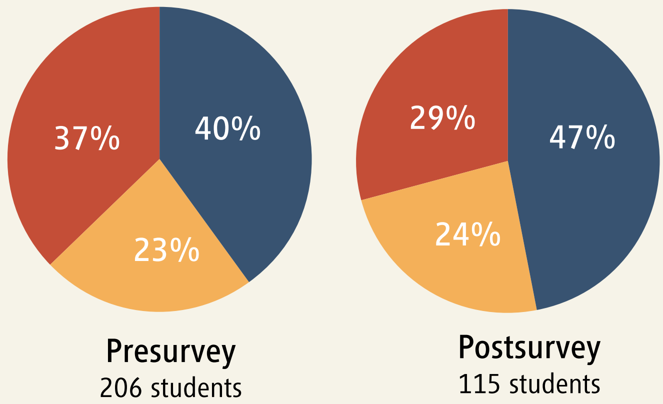
Another means of spreading the word are Darwin celebrations on campus that coincide with the biologist's 12 February birthday. The College of Charleston started a "Darwin Week" 6 years ago to combat attempted antievolution "mischief" in the state legislature, says Dillon. The University of Alabama is having its first "Darwin Day" this year, and Provine says Cornell is considering starting one. The University of Tennessee, Knoxville, has celebrated the great man's birthday since 1997, when Pigliucci sought to rebut an "equal time" bill being considered in the state legislature.

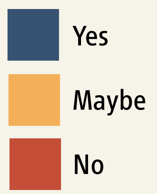
"The first time we offered Darwin Day, a local TV station made fun of the whole thing by taking shots of chimps at the zoo," recalls Pigliucci. Ecology grad student Marc Cadotte says the media have moved on but that quite a few local high school teachers are attending the Darwin Day teachers' workshop: "It's an encouraging sign that our activities are making a difference."

–CONSTANCE HOLDEN

A Survey of Student Attitudes

The question: Do you accept that modern man and modern great apes had a common ancestor several million years ago?





Resistant to change. Pre- and postcourse surveys of biology students at West Virginia University, Parkersburg, indicate that human origins are a sticking point for those who embrace the Bible.

SOURCE: EDWARD L. CRISP/WEST VIRGINIA UNIVERSITY, PARKERSBURG

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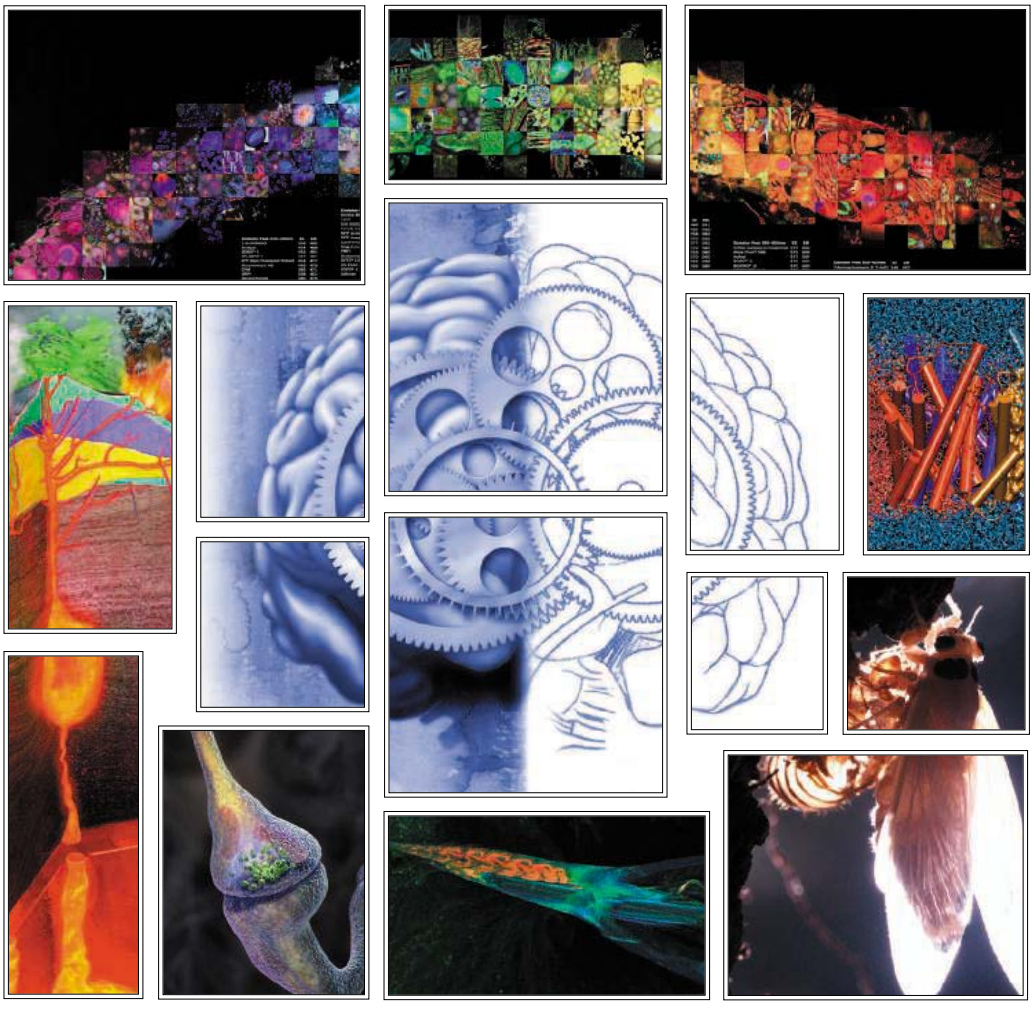

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EDITED BY YUDHIJIT BHATTACHARJEE

Pioneers

DETAIL, MORE DETAIL. For more than 2 decades, photomicroscopist Dee Breger of Drexel University in Philadelphia, Pennsylvania, has used artistic photographs from scanning electron microscopes (SEMs) to lure the public into learning about science. "It's a bait and switch," says Breger (left, top). "You grab someone's attention with a pretty image, and you tell them what it is." Last fall, she did one better: She offered an hourlong guided session on an SEM as part of an auction to raise money for educational software.

The auction, organized by Galaxygoo, a San Francisco nonprofit, featured works of art inspired by science, including Breger's own SEM image of a penguin feather. The SEM session was bought on eBay last month by Aaron Messing (center), a 65-year-old amateur microscopist, for $153.75. Messing, who has eight light microscopes at his West Orange, New Jersey, home, says the 2-million-fold magnification was too good to pass up.

Messing is preparing for his session by reading the microscope's lengthy user's manual. He hopes to study "some state-of-the-art nano-items" borrowed from Drexel's College of Engineering and avoid "a nice little exercise that is cute but not meaningful."

MOVERS

CROSSING OVER. In 2000, Aristides Patrinos brokered a truce between a U.S. government project to sequence the human genome and a competing, private effort led by J. Craig Venter. Now, the 59-year-old engineer-turned–science administrator is leaving the Department of Energy after 3 decades to run a company founded by Venter. As president of Synthetic Genomics in Rockville, Maryland, Patrinos will work with companies to develop made-to-order microbes that can produce ethanol or hydrogen, eat oil, or sequester carbon.

"He's got the respect of the entire government and scientific communities," says Venter, a former government employee himself who has since founded a string of companies and research organizations. Patrinos is also a star in Greece, the native country of his parents: Last year, he finished fifth in a poll by an Athens newspaper for Person of the Year.

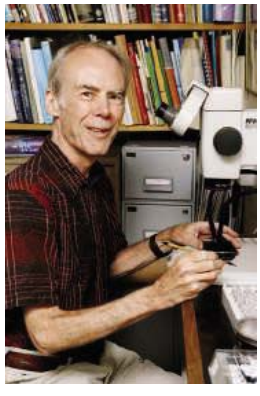
AWARDS

SPANISH ECOLOGY PRIZE. Biological oceanographer Paul Dayton is the inaugural recipient of a new ecology prize awarded in memory of Spanish ecologist Ramón Margalef i López. Dayton, a researcher at the University of California, San Diego, known for his work on coastal, estuarine, and Antarctic habitats, received the $120,000 award at a ceremony in Barcelona last month. The prize is funded by the government of Catalonia.

DEATHS

IDEAS AND MORE. Great Britain's Nicholas Shackleton started out studying physics, switched to measuring minuscule isotopic differences in microscopic bits of ocean mud, and ended up establishing the metronomic qualities of climate change. His death on 24 January, at age 68, leaves a hole in the field of paleoceanography that he pioneered with the understanding that ice ages fluctuate with the rhythmic variations in Earth's orbit.

"He was full of ideas, but he followed up with measurements," says marine geochemist



Wallace Broecker of Lamont-Doherty Earth Observatory in Palisades, New York. "The only competition for his work was the clarinet."

Shackleton, who retired from Cambridge University in 2004, amassed a world-class if not unique collection of historical clarinets, published scholarly papers on the instrument, and played it himself. "In a sense," says Broecker, "he was just as much an explorer" as his distant relative, Antarctic adventurer Ernest Shackleton.

Two Cultures >>

GENOMIC ADVANCE. Working with Princeton University biologist Bonnie Bassler, choreographer Liz Lerman has developed a multimedia production that mixes dance with scientific imagery to explore the impact of genetic research on society. This photo shows Gregor Mendel (acted by Ted Johnson) leading a female protagonist (Margot Greenlee) on a journey retracing evolution's footsteps. The production, titled *Ferocious Beauty: Genome*, premiered at Wesleyan University in Middletown, Connecticut, last week.

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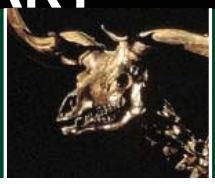

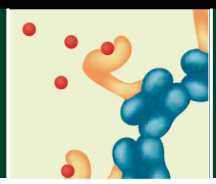

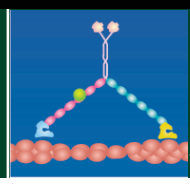

LETTERS

edited by Etta Kavanagh

Peer Review and New Investigators

IN HIS LETTER "REVAMPING NIH STUDY SECTIONS" (6 JAN., P. 36), J. LENARD asserts that removal of assistant professors from review panels "to their own great benefit" would "immediately improve" the quality of review and, presumably, "correct some of the distortions." The only such distortions specified are "political" and "subculture-sensitive" biases. It is not clear why less experienced scientists would be more biased in this regard; one would imagine that they have much less in the way of entrenched bias. The advantages of young scientists participating on a review panel are obvious. The best way to improve one's success in grant writing is to read many proposals and to experience firsthand the subtle dynamics of the review panel. Balancing these advantages against the time and energy subtracted from the scientist's own research is best left up to the individual.

"The best way to improve one's success in grant writing is to read many proposals and to experience firsthand the subtle dynamics of the review panel."

—Taffe

Regarding "distortions," the average age upon obtaining the first R01/R29 award reached 42 years of age in 2002, up from 37 in 1980 (*1*). The proportion of competing research grants awarded to scientists under 35 was 4% in 2001, down from 23% in 1980 (*2*). Declines for young/new investigator success on these and other measures have been uninterrupted for two decades of increasing NIH funding. Many NIH initiatives such as the R29 program, the "new investigator check box," revisions to review criteria/guidance, and the recent launch of a Web page on New Investigators (*3*) suggest that NIH considers the ongoing declines in young/new investigator success to be a "distortion" of significant importance.

The Center for Scientific Review (CSR) databook (*4*) reports that 26% of standing, and 28.5% of ad-hoc, members of panels were 45 years of age or younger in 2004. The CSR report also confirms that new investigators receive lower median priority scores than experienced investigators and are less likely to submit A1 and A2 revisions, as well as testifying to the substantially improved cumulative funding probability with successive revisions. The director of CSR, Toni Scarpa, is to be congratulated in his efforts to revamp peer review, his desire to make revisions based on review data, and his clear focus on the new investigator as a priority ("Peer review at NIH," Policy Forum, 6 Jan., p. 41). I hope that in this process, he considers the role of career rank quite closely.

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Keeping the DSCOVR Mission Alive

THE TITLE OF *SCIENCE*'S ARTICLE ABOUT NASA'S decision to cancel the Deep Space Climate Observatory (DSCOVR) satellite mission, "NASA terminates Gore's eye on Earth" (ScienceScope, A. Lawler, 6 Jan., p. 26), was misleading. This title trivializes the real nature of the mission and obscures the fact that DSCOVR is not the same as the Triana mission promoted by then Vice President Gore. The Triana concept was to provide the public (via the Internet) with a continuous, real-time image of the entire, sunlit Earth, essentially a TV camera in space. DSCOVR is a high-priority, peer-reviewed scientific mission, conceived and developed by a team of experts.

In 1998, NASA issued a request for information to the science community regarding utilization of the L-1 Lagrange point between Earth and the Sun, from which the entire sunlit hemisphere of our planet can be continuously observed. Our team responded by recommending broadband and high-resolution, spectro-radiometric measurements that would improve understanding of the solar/infrared energy balance (*1*) for the Earth system as well as of atmospheric composition and dynamics. Importantly, these observations would provide calibrations and integral constraints for all satellites in geostationary and low Earth orbit because they all are at times in view from L-1.

Our proposal was selected by NASA after rigorous scientific and technical reviews. Solar activity observations were added at NASA's request to satisfy scientific needs and NOAA's operational requirements, for space weather monitoring. DSCOVR is firmly based on the ideas developed by the science team. The transmission of live images of Earth added to the educational outreach component of the mission but was by no means the primary objective.

Many scientists, both in the United States and abroad, view DSCOVR as one of NASA's most important and innovative Earth science missions. The satellite has been built and could still be launched in time to provide synergistic data coincident with current and future orbiting systems. It offers great potential both as a source of fundamental scientific observations and as a pioneering Earth sciences mission from deep space.

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How to Measure National Stereotypes?

BECAUSE IT IS PARTICULARLY DIFFICULT TO EVALuate the accuracy of national stereotypes, the Report by A. Terracciano *et al.* ("National character does not reflect mean personality trait levels in 49 cultures," 7 Oct. 2005, p. 96) examining the relations between ratings of national character and ratings of individuals in 49 different cultures represents quite a technical achievement. Studies of stereotypes usually suggest that stereotypic beliefs contain a kernel of truth: The perceived differences between groups do in fact exist, but they are smaller than the stereotype would suggest (*1*, *2*). Terracciano *et al.* instead found that, on average, there was no relation between national stereotypes and self and other descriptions. Some methodological weaknesses of their study must be considered, however.

One issue is their almost exclusive reliance on college student samples. Although there is some evidence that cross-cultural comparisons between college students may generalize to broader populations (*3*), there is also substantial evidence that findings with college students frequently do not so generalize (*4*). These findings do not invalidate college student samples as representations of broader national populations, but neither do they justify assuming college students provide an acceptable proxy for the population as a whole.

A second issue is whether the authors have provided a sufficient evaluation of national character. The authors reduce national character to personality traits. This ignores other potential elements of stereotype, most particularly differences in values, beliefs, or perceptions that are not adequately included in the measures used in this study.

Finally, Terracciano *et al.*'s measures of perceived national character were the mean ratings of the culture by members of that culture. Stereotypes are usually defined in terms of perceptions of the target group by outside observers. Moreover, their measure of actual national character was the mean ratings of oneself or a significant other. In other words, the measurement of national character was based on the ratings of a culture, whereas the measurement of actual character was based on the ratings of a person. The contexts of the two kinds of assessments were quite different and potentially not comparable.

It is increasingly evident that context is an important contributor to outcomes on rating scales (*5*). There is even evidence that cultural differences by themselves can produce differences in the context of the measurement (*6*). A person familiar to the respondent will likely be evaluated in relation to other individuals familiar to the respondent, while a person asked to rate the culture will rate it in relation to other cultures. It is not surprising then to find that these ratings were on average unrelated to ratings of the country's national character.

It is possible that there really is no relation between national stereotypes and actual behaviors. One must wonder, however, what is the source of the variability in the ratings of cultures. Why, for example, do the German Swiss believe they are so conscientious? Even more curious is why Indonesians and Chileans accept that they are not. It seems likely that when asked to rate themselves on conscien-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

tiousness, German Swiss evaluate themselves in light of those around them. A more definitive test would be to have the German Swiss rated by members of other cultures, but then that is presumably the kernel from which cultural stereotypes germinate in the first place.

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CONSISTENT WITH A LONG-HELD VIEW IN SOCIAL psychology, A. Terracciano and colleagues claim that national stereotypes lack accuracy ("National character does not reflect mean personality trait levels in 49 cultures," Reports, 7 Oct. 2005, p. 96). Although it is possible that their findings demonstrate people's inability to discern the attributes of their own groups, three alternative explanations need to be considered.

First, the criterion scores, which were obtained from responses on a personality inventory [the Revised NEO Personality Inventory (NEO-PI-R)], were less variable than the stereotype scores, which were obtained with a new instrument [the National Character Survey (NCS)]. Arguably, the greater length of the NEO-PI-R facet scales (eight items) relative to the NCS scales (one item) contributed to this difference. Furthermore, the nonrepresentative sampling of respondents could have reduced the variability of the criterion scores, as college students tend to share similarities in different cultures.

Second, the similarity of the sample profiles was assessed with intraclass correlation coefficients (ICCs). ICCs are used for dyadic data that cannot be sorted. When judgments are correlated with criteria, Pearson correlations are more appropriate. These indices are only sensitive to profile similarity, not to differences in variability.

Third, national characteristics and stereotypes can be specific. The Japanese may be uniquely characterized by their deference, whereas people from the United States may be known for their materialism. If so, measures of profile similarity gravitate toward zero as a function of profile length.

Failures to reject a null hypothesis are usually not newsworthy. A typical response is to design a study to minimize contaminating effects. Here, however, the embrace of the null hypothesis is also a conceptual surprise. Historically, research on the five-factor model of personality has been predicated on observer agreement, where agreement was thought to imply accuracy. Now, the role of observer agreement is to signal inaccuracy. It is certainly possible that perceptions of nations are qualitatively different from perceptions of individuals, but to find out we need a process model that specifies how people judge national character and how they might agree without being accurate.

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Response

WE AGREE WITH MCGRATH AND GOLDBERG THAT national stereotypes include more than national character, and beliefs about national differences in appearance, attitudes, or athletic abilities may or may not be accurate. Our study focused on personality traits, which seem to define the core of national character. To the extent that the five-factor model (FFM) is comprehensive, our National Character Survey (NCS) measured key features of national character, and we found no evidence for a kernel of truth in these stereotypes.

Student samples may or may not generalize to

the general population, but there is reason to think they did in this instance. Previous work comparing personality ratings from students and adults showed generalizability (*1*, *2*). In our study, the self-report criterion included data from adults, and in cultures where personality profiles were based solely on adult self-reports, we found no support for the accuracy of national character stereotypes. Finally, national character ratings by adults in Ethiopia, Italy, and the Philippines (*3*) agreed with students' NCS rating. All the available data suggest that the age of the raters did not affect the outcome.

McGrath and Goldberg suggest that our "national character was based on the ratings of a culture" and thus we compared cultures with people. In fact, the NCS asked respondents to describe the typical member of their culture. Italian raters, for example, were asked to respond to the stem "Italians are likely to be …" Factoring NCS responses led to the familiar FFM, as one would expect ratings of persons to do (*4*). Personality and national character assessments were fully comparable; they were simply different.

McGrath and Goldberg also raise the issue of changing frames of reference. There is no evidence that these compromise personality ratings. The reference group effect would tend to eliminate any cross-cultural differences in personality traits and render them meaningless [see note (*27*) of our Report], but our aggregate scores varied systematically across cultures, formed clear geographical clusters, and showed meaningful correlations with culture-level variables (*1*). Thus, the reference effect cannot explain the failure to find correlations with NCS scales.

Finally, McGrath and Goldberg also suggest that a more definitive test of stereotype accuracy would employ out-group judgments. However, the literature (*3*, *5*, *6*) and our own data (*7*) indicate that out-group ratings of national character are very similar to in-group ratings, at least between neighboring cultures. Given such similarity and the simple fact that people presumably know members of their own culture better than foreigners do, it is not clear how out-group ratings would be accurate.

Krueger and Wright propose alternative explanations for our finding that national stereotypes are inaccurate, but none seems justified.

The first concerns reliability. The eight-item NEO-PI-R (*8*) scales are presumably more reliable than the single-item NCS scales. Greater reliability means less random error, and that should increase, not decrease, the variability of the NEO-PI-R scales across cultures. The greater variability of the NCS scores that we in fact observed is more likely due to the exaggeration that is characteristic of stereotyping.

Although the observer rating criteria were obtained from college students, the inaccuracy of stereotypes was confirmed by self-report data [our Report; (*2*)] from samples of adults as well as students.

Krueger and Wright note that ICCs are used for interchangeable dyadic data. They are also used to assess absolute agreement that takes into account the means and variances of two sets of measures. Pearson correlations are sensitive only to the shape of a profile; they ignore differences in elevation. Imagine two sets of 30 scores with perfectly parallel profiles but with a constant difference of 10 *T*-score points. The Pearson correlation would be 1.0, suggesting perfect agreement despite the large mean differences. The ICC method we used would give a much lower or negative coefficient because of its sensitivity to the 10 *T*-score points difference, correctly showing that the profiles are substantively very different. However, we reanalyzed the data using Pearson correlations and found very similar results, with median correlations of 0.08 and –0.01 for the observer rating and self-report data, respectively.

Krueger and Wright suggest that stereotypes may be specific for different cultures and their effects may be diluted by analyses of all 30 traits in each culture. Although some stereotypical traits may be more salient than others, the aggregate NCS ratings were highly reliable for all five factors and 30 facets, indicating that the raters

shared perceptions of the typical member of their culture on most traits.

Null findings are not newsworthy if they are based on a weak study. But our project used data from over 40,000 respondents in 49 cultures, employed a comprehensive selection of personality traits, examined agreement both across and within cultures, and replicated the null findings using two methods of assessing personality. Agreement between observers was taken as evidence of accuracy [e.g., (*1*)] and the failure to find agreement was considered evidence of inaccuracy.

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TECHNICAL COMMENT ABSTRACTS

Comment on "Zircon Thermometer Reveals Minimum Melting Conditions on Earliest Earth" I

Andrew Glikson

Watson and Harrison (Reports, 6 May 2005, p. 841) proposed a model for early Earth magmatism based on crystallization temperatures of Hadean zircons. However, detrital zircon populations are skewed relative to the composition of their source terrains, Archaean isotopic and geochemical mantle signatures preclude reincorporation of Hadean continental crust into the early mantle, and the effects of early impacts should be considered.

Full text at www.sciencemag.org/cgi/content/full/311/5762/779a

Comment on "Zircon Thermometer Reveals Minimum Melting Conditions on Earliest Earth" II

Allen P. Nutman

Watson and Harrison (Reports, 6 May 2005, p. 841) interpreted low temperatures (~700°C) for Hadean zircons as evidence of the existence of wet, minimum-melting conditions within 200 million years of solar system formation. However, high-temperature melts (~900°C) are zircon undersaturated and crystallize zircon only after substantial temperature drop during fractional crystallization. Zircon thermometry cannot distinguish between low- and high-temperature Hadean igneous sources.

Full text at www.sciencemag.org/cgi/content/full/311/5762/779b

Response to Comments on "Zircon Thermometer Reveals Minimum Melting Conditions on Earliest Earth"

E. B. Watson and T. M. Harrison

The mean crystallization temperature of Hadean zircons based on titanium content is ~680°C. This value corresponds to the temperature of wet minimum melting in present-day crust. The low variance of the temperature distribution (±25°C) also points to Hadean zircon growth under conditions that were highly reproducible and thermally regulated. Eutectic-like melting is particularly capable of providing such regulation and is consistent with Hadean zircon growth during wet crustal fusion.

Full text at www.sciencemag.org/cgi/content/full/311/5762/779c

PUBLIC HEALTH

Chicken Monster or Chicken Little?

Steven M. Wolinsky

This is a scary time to be a chicken. Free-range lives on traditional backyard poultry farms have quickly been replaced by large-scale confinement in a crowded, ghetto-like environment. Feeding operations in such compressed living conditions stress the chickens' immune systems and make them susceptible to infectious diseases. An outbreak of highly pathogenic avian influenza in a poultry farm is the harbinger of more than a few fowl deaths—agricultural authorities will cull entire flocks of chickens within a 2-kilometer radius of where a sick or dead bird is found.

With severe outbreaks of H5N1 (*1*) avian influenza in aquatic birds from Asia to Eastern Europe—and tentative leaps into other species—it is a scary time to be a human as well. Just how scary is the alarming message Mike Davis seeks to convey in his short new book, *The Monster at Our Door: The Global Threat of Avian Flu*. He argues that a biomedical pandemic akin to the 1918 influenza virus beckons, driven by the greed of multinational corporations, poverty, corrupt governments, and raw political ambition.

Davis—a social commentator, professor of urban and environmental history at the University of California, Irvine, and author of the best-selling *Ecology of Fear* (*2*)—chronicles the course of human events that have conspired to create a fertile breeding ground for the next influenza pandemic. At times the book reads too much like a thriller, and the author could have delved further into the underlying epidemiological and evolutionary dynamics of influenza. Its strength, though, lies in its clear—and frightening—illustration of the stopgaps for containment.

After the 1918 H1N1 influenza pandemic (which killed more than 20 million people), the subsequent, less deadly experiences with 1957 H2N2 and 1968 H3N2 left "an ambiguous legacy." Davis says the relatively mild effects of the latter influenzas relaxed fears about future pandemics but nonetheless exposed problems with the "profit-driven vaccine marketplace." Hong Kong in 1997 juxtaposed a dense urban population, live-poultry markets, and the falcated teal ("the duck of the apocalypse") in the city's Mai Po and Deep Bay marshes. Under those circumstances, H5N1 avian influenza jumped directly from bird to man with few molecular modifications. Officials ultimately responded with a frenzied, massive culling of the ducks, geese, chickens, and wild birds.

Hong Kong researchers gained a sense of false security when their "'attempts to transmit experimentally a number of avian virus subtypes directly to humans were not successful'"; they concluded the species barrier was "insurmountable" (only the H1, H2, and H3 serologic subtypes are known to have caused human pandemics). Nevertheless, in 1999, H9N2 avian influenza (a direct decedent of the 1997 H5N1 avian influenza) infected several people in the Chinese province of Guangdong. A survey of viruses isolated from ducks in the live-poultry markets of Shantou, Guangdong, revealed a staggering finding: "almost 500 distinct strains of influenza." Less than six years later, in 2003, H5N1 avian influenza was ravaging the aquatic birds that served as its natural host in earnest. Researchers linked the outbreak of the H5N1 superstrain (genotype Z) to "a clandestine and misguided vaccination campaign" in southern China (begun after the 1997 outbreak in Hong Kong) that enabled its rapid dissemination (*3*).

The Monster at Our Door
The Global Threat of Avian Flu
by Mike Davis
New Press, New York, 2005. 220 pp. $21.95, C$26.95, £12.99. ISBN 1-59558-011-5.

Smart chickens would flee if they could.

With poultry becoming the principal source of the developing world's meat consumption, agribusiness has a large stake in the crisis. Davis points out that multinational cartels like Arkansas-based Tyson Foods and Bangkok-based Charoen Pokphand (CP) embraced intensive farming and shunned oversight of their product. He suggests that with CP's incredible economic power and political clout at home and abroad—it donated to both the Bush senior and Clinton presidential campaigns—the company avoided the government-mandated cull of the open-air flocks. Meanwhile, many poultry farmers, offered too little money to cull their birds, suppressed information about possible infections. Small farmers also tended to conceal their valuable fighting cocks.

"CP claimed that its industrialized, enclosed farming system was virtually impregnable to viral outbreaks and epidemics," Davis writes. Nevertheless, when chickens began dying across Thailand in 2003, the CP chicken processing plants worked overtime "rushing to process the chickens before getting any veterinary inspection." And in China, transparency of reporting and disease surveillance was essentially nonexistent. In the end, H5N1 avian influenza became "ecologically entrenched" in waterfowl throughout Asia.

Davis aptly describes the impact of the pernicious epidemic but does not explain its biology clearly enough. Dissecting the virus is critical to understanding its potential scope—the variability that originates with high viral replication and mutation rates, which is then shaped by recombination and gene reassortment. Davis gives passing reference to the connection between the epidemiological and immunological forces that along with genetic variation of the influenza virus drive the waves of infection across the globe. The evolutionary and epidemiological dynamics of the more common influenza A is the key reason why we must formulate a new influenza vaccine each year.

Aquatic birds do pose a real threat. They are the natural reservoir for influenza A, and because they are infected with a variety of antigenic types, they can provide the venue for novel combinations of H and N genes to create an antigenic shift that transcends our collective immunological experience. Without this prior immunological knowledge, an anticipatory vaccine seed stock, and efficacious drugs, a population is at risk for a pandemic.

Davis is among those who worry that H5N1 influenza will cross over from birds with the very same ferocity as the 1918 H1N1 strain. It is true that viruses jump between species. Many viruses, including the human immunodeficiency virus (HIV), monkeypox, and the SARS-coronavirus, have made the leap from other animals into

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humans. Such infections across species boundaries can lead to a dead end, with the virus not being readily transmissible from human to human. (This might explain, for example, the scarcity of the HIV N group.) On the other hand, successful human-to-human transmission of H5N1 avian influenza would create a global crisis. Not knowing which particular genetic variant will sustain human-to-human transmission compromises our ability to formulate a vaccine in advance—and this is just one of the many practical and immunological challenges for developing a vaccine. Nonetheless, contrary to Davis's assertions about natural selection, the leap between species does not necessarily "favor increased virulence" in the new host. Although many people infected with H5N1 have died, the number of asymptomatic cases is unknown.

In the 1980s, the Institute of Medicine warned that "the United States was ill-prepared to face the threat of emergent diseases." The breakdown in the public health infrastructure, Davis argues, was colliding with "radical changes in disease ecology being wrought by globalization." Few pharmaceutical companies still manufacture vaccines, and the ones that remain have been plagued by production difficulties. Without surge capacity, our ability to stockpile oseltamivir to prevent and possibly treat infection is also impeded. (Then again, an H5N1 influenza isolated from pigs in Java was resistant to the drug, a likely consequence of its inappropriate use.) Davis claims that the government misspends its money on biodefense initiatives to protect us from biological threats that are unlikely to occur. In his view, governments and industry—each for their own selfish reasons—have formed a confederacy of dunces. Only lone scientists, with their tireless work, have captured the author's admiration and escaped his condemnation. To Davis, the mantra of epidemiologists and basic scientists alike is loud and clear: we are not prepared for the next pandemic.

No doubt that avian flu is a threat to guard against. But the deeper question—which remains unanswered in *The Monster at the Door*—is how do we accurately gauge the risk. For chickens the risk is substantial, and, granted free will and free range, it would make sense for them to flee the routes followed by migratory birds infected with H5N1 influenza. For us, the challenge is to achieve a proper balance between the dire warnings of Chicken Little and the folly of playing ostrich.

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PHILOSOPHY OF SCIENCE

Wallowing in the Wastebin

Douglas Allchin

In science (so the saying goes) "man proposes, nature disposes." Many theories thus end up in the wastebin. We no longer talk of phlogiston, caloric, electrical fluid, pangenes, bodily humors, or immobile continents. Such historical errors puncture easy interpretations of cumulative scientific progress. For John Losee, in his provocatively titled *Theories on the Scrap Heap*, they are also prime occasions to consider how scientists evaluate theories. In a clever turnabout, he asks not how investigators establish evidential support for theories but why they find certain theories inadequate, even if once widely accepted.

Theories on the Scrap Heap
Scientists and Philosophers on the Falsification, Rejection, and Replacement of Theories

by John Losee

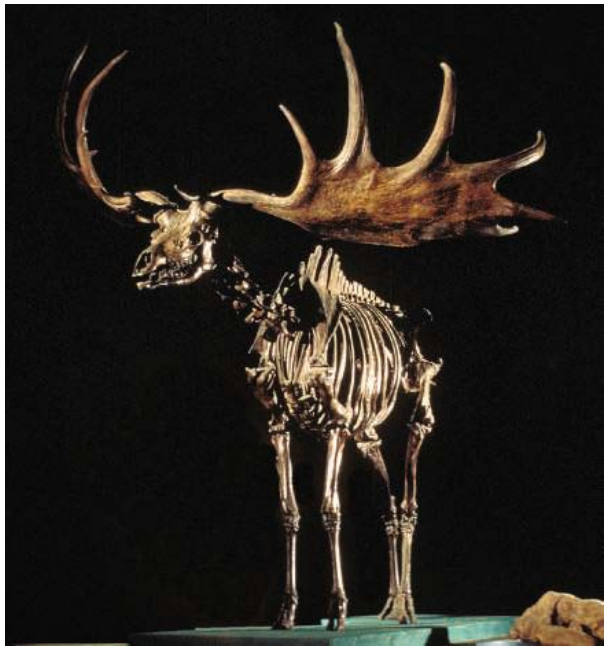
University of Pittsburgh Press, Pittsburgh, PA, 2005. 216 pp. $24.95. ISBN 0-8229-5873-2.

Losee, an emeritus professor of philosophy at Lafayette College in Pennsylvania, feels that any account should be responsible to history. Prescriptive ideals should, as he has argued in earlier works, give way to a descriptive philosophy of science. Any proposed standard for evaluating theories should have proven effective in the past.

Respect for historical evidence can yield surprising results. For example, how important are confirmations of novel predictions? How do they fare relative to post hoc accommodations? One can easily cite a handful of dramatically vindicated predictions: Halley's comet, Mendeleev's new elements, Adams's and Leverrier's eighth planet. Some folkloric histories, however, are misinformed: neither Einstein's gravitational bending of light nor Poisson's bright spot as proof of the wave theory of light had the historical significance they are often granted in retrospect. Contrary to widespread stories, William Harvey did not even predict the capillaries later "confirmed" by Marcello Malpighi's observations. In other cases, such as Bethe's theory of solar energy production and Agassiz's glacial explanation of erratics, accommodation of available evidence alone seemed sufficient.

Moreover, successful prediction does not guarantee correctness. Belief in phlogiston led Priestley to predict that the substance produced when metals are dissolved in acid would act like charcoal (in today's terms, hydrogen is a reducing agent). Other phlogistonists predicted that electricity should reduce calxes to their metals. Dalton and Gay-Lussac each used the concept of caloric (heat as a fluid) to predict that all gases should share the same rate of expansion as temperature rises. Ptolemy's Earth-centered cosmology still predicts eclipses and the positions of the planets. Yet all three theories now lie abandoned in disrepute. Using past experience as a benchmark, "it would seem that the predictivist thesis is false."

Such cases may seem to warrant another common belief about science: one can never prove a theory, but one can disprove it (typically with a single, well-framed study). Here, Losee tackles Popper's notion of falsification, which is crudely expressed in the proposal-disposal aphorism. To gauge current views



Tales of Irish antlers. Supporters of orthogenesis presented the increase in antler size and subsequent extinction of the "Irish elk" *Megaloceras* as prima facie disconfirming evidence for the theory of natural selection. Other evolutionary biologists interpreted the antlers as adapted for display in open environments but nonadaptive in thick forests that arose as glaciers retreated.

CREDIT: CHIP CLARK/NATIONAL MUSEUM OF NATURAL HISTORY, SMITHSONIAN INSTITUTION

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about falsification, I surveyed the use of the term in *Science* over the past 10 years (*1*). Three-fifths of the cases referred to misconduct: falsified data or research reports. Of the remainder (46), three-fifths appealed to falsifiability as a hallmark of science or of proper rigor in science (from archaeology and chemical bonding to climate change and paleontology). For example: "Science is based on the falsification of hypotheses." Scientists "work late into the night in order to destroy or falsify another scientist's hypothesis." Researchers who fail to present falsifiable theories are "not playing the game." A theory that cannot predict falsifiable hypotheses is not "sophisticated enough."

In 16 cases, single findings were interpreted explicitly as falsifying some claim. A news item noted that critics of teaching evolution frequently apply such stark falsificationist views. In far fewer (three) cases, authors deemed such judgments too simplistic. One cautioned against rejecting a theory prematurely. Losee agrees, echoing a decades-old consensus among philosophers of science (*2*, *3*). He details through historic cases how one set of negative results is rarely decisive, except for quite low-level hypotheses. Rather, researchers typically finesse the evidence by redefining terms, modifying theories, restricting their scope, or even tolerating unresolved anomalies. Effective reasoning seems to integrate both counterevidence and evidence, and weaker theories wane.

Falsification may also be construed as a methodological guide: guard against error through rigorous self-criticism. Indeed, Popper profiled his "severe tests" as self-referential. Ironically, appeals to falsification in this journal invariably seem to target critics instead. Losee thus opens his book appropriately by characterizing falsification as foremost a "rhetorical strategy," not a touchstone of science. The basic lessons about reliability may be better, if less dramatically, expressed as the significance of empirical import (testability), systematic review of possible sources of error, and thoroughly ruling out alternative explanations (*4*).

Losee's discussion, although offering students a foundational introduction, may strike well-informed readers as dated and conspicuously incomplete. The author leads us to the brink with some tantalizing puzzles. Unanticipated regularities may be predicted by strictly false theories, but how? Theories based on nonreal entities may be empirically successful. Lavoisier could help develop calorimetry, even though the caloric it purported to measure seems illusory. To interpret these paradoxical achievements, one may reconceptualize theories more modestly, as not universally applicable. Philosophers have crafted an alternative based on local, delimited models, which may overlap and possibly even conflict (*5*). They can thereby articulate how to deal with exceptions without sacrificing the ideal of invariant causal generalizations (*6*). One need not jettison phlogiston as an inviable theory. Alternatively, one may accept it as a truthful model, provided one frames its context appropriately. As Losee acknowledges, we still accept Newtonian mechanics, knowing full well we cannot apply it to very light, very fast bodies. Rather than judge theories wholesale, a reflective investigator will seek a more nuanced framework for focusing on and managing errors (*7*, *8*).

Another puzzle is how eminent scientists can disagree—what separated Newton and Leibniz or Bohr and Einstein. Losee describes how individuals may differ due to principled themes or biographical "idiosyncracies." If so and if the scientific enterprise is collective, how do divergent interpretations interact (and possibly become reconciled)? How do researchers accommodate one another's blind spots? How might diversity among practitioners foster more fruitful checks and balances? Readers will want to complement Losee's account with recent epistemic analyses of the social structure of science (*9*–*12*).

Theories on the Scrap Heap provides a lucid, nicely consolidated introduction to the appraisal of scientific theories. Nonetheless, many perspectives in the past several decades—cognitive, rhetorical, gendered, and cultural—extend far more deeply our appreciation of the roots and remedies of error.

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BROWSING

Vanishing Act. Art Wolfe. Text by Barbara Sleeper. Bulfinch, New York, 2005. 144 pp. $50, C$67. ISBN 0-8212-5750-1.

In their efforts to capture wildlife on film, most photographers strive to make the animals stand out from their surroundings. In this series of 101 photographs, Wolfe instead documents how his subjects disappear into their environments. Some are camouflaged by their coloration or patterning, others rely on their shapes and behaviors, and many combine several approaches to deceive predators or prey. Because photographs cannot capture all of the distractions of the landscape, Wolfe uses depth of field, the placement of his subjects within the frame, and inclusion of bigger or brighter distractions to make his images visually challenging. The book is filled with examples of animals—mammals, birds, reptiles, amphibians, fish, insects, and crabs—that seem to vanish in plain sight, such as the Elliot's chameleon, *Chamaeleo ellioti* (above), photographed in Rwanda's Parc National des Volcans. Concise descriptions by Sleeper provide details about the organisms and their ecology.

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ETHICS

Incidental Findings in Brain Imaging Research

What should happen when a researcher sees a potential health problem in a brain scan from a research subject?

Judy Illes,[1,2] Matthew P. Kirschen,[1–3] Emmeline Edwards,[5] L R. Stanford,[6] Peter Bandettini,[7] Mildred K. Cho,[1,4] Paul J. Ford,[8] Gary H. Glover,[2] Jennifer Kulynych,[9] Ruth Macklin,[10] Daniel B. Michael,[11] Susan M. Wolf[12] and members of the Working Group on Incidental Findings in Brain Imaging Research[13]

Research imaging studies have provided a steady stream of fundamental knowledge about the relation between brain and behavior in health and disease. Recent reports of clinical findings detected incidentally in this research (*1–3*), however, have created interest in the implications and ethics of how these findings are handled. We define incidental findings as observations of potential clinical significance unexpectedly discovered in healthy subjects or in patients recruited to brain imaging research studies and unrelated to the purpose or variables of the study. We believe that all investigators engaged in brain imaging research should anticipate incidental findings in their experimental protocols and establish a pathway for handling them. The central issues for consideration are how to protect subject welfare and research integrity while appropriately addressing investigator responsibility, subject expectations, informed consent, professional training of the research team, and the financial cost of following up on incidental findings. Protecting human subjects is of paramount importance.

This article summarizes the views presented at a workshop sponsored by the U.S. National Institutes of Health (NIH) (*4*) and ongoing work, but it does not reflect endorsement by or an official position of the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute on Drug Abuse (NIDA), the National Institute of Mental Health (NIMH), NIH, or any other Federal agency. It is intended to advance discussion of the issues only (*5*). Any future official recommendations on incidental findings should promote trust in research without unduly encumbering the scientific process.

“Any future official recommendations on incidental findings should promote trust in research without unduly encumbering the scientific process.”

Published data indicate that clinically significant and identifiable neuropathologies occur in 0.5 to 2% of the population (*6*). There have been some reports of higher rates of incidental findings with varying degrees of clinical significance (*1–3*, *7*, *8*). Low rates of clinical disease have also been reported by the Central Brain Tumor Registry of the United States (*9*). Taken together, these data raise the possibility of a high rate of false-positives in incidental findings.

Issues concerning communication focus on whether incidental findings in research should be disclosed to subjects at all and, if so, what are the obligations of researchers to communicate them, when, and to whom. The discussion is made especially complex by the absence of professional guidelines and the current landscape of imaging investigators that includes undergraduate and graduate students, fellows, and M.D. and Ph.D. investigators. A practical obstacle in establishing guidelines is that there are no data on the usefulness of brain imaging as a screening tool in asymptomatic individuals, particularly the typically lower-resolution and contrast magnetic resonance (MR) images collected for research.

The majority of the working group (*10*) felt that a research protocol that provides for disclosure of suspicious incidental findings to subjects is ethically desirable. This view is based on researcher obligations to respect subjects' autonomy and interests, demonstrating reciprocity when subjects agree to participate in studies by communicating a finding that may have a health impact (*11*). This view is also informed, in part, by results of a recent study (*12*) in which subjects who had previously participated in brain imaging studies were queried for their expectations about incidental findings. Whether scanned at an imaging facility affiliated with a medical center or at a nonmedical site associated with a university psychology department, subjects reported that they expected an abnormality to be detected if present. An average of 97% reported that they wanted a finding to be disclosed to them regardless of its potential clinical significance. Much remains to be learned about the source of subjects' expectations in such studies, including their understanding of informed consent.

The potentially harmful consequences of false-positive reports on normal volunteers have not been explored. Some members of the working group felt that the potential of false-positives rendered it unwise to communicate all but the most certain incidental finding.

Wide variability exists in both when and how incidental findings are handled for the estimated tens of thousands of human subjects involved in imaging research per year. In one survey of MR imaging laboratories, 36% reported that all their research scans are read by a neuroradiologist and findings disclosed, 47% only when a suspicious finding is detected, 4% depending on type of study, and 13% not at all (*2*). Some research centers are not associated with clinical facilities, and anomalies may be detected by and reported to the subject by nonphysicians. In these cases, the subjects are informed that the scan is a research scan, that the researchers are nonphysicians, and that they are not qualified to evaluate any anomalies detected. Data of potential medical significance may be made available to a physician if the subject chooses, but should never be disclosed without explicit authorization of the subject or a surrogate when the subject is a minor or an adult without decisional capacity.

Vulnerable populations and subjects without a primary-care physician or without medical insurance may need extra assistance in

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identifying avenues for follow-up consultation. A minority of the group felt it was not ethical to leave all of the responsibility for follow-up to the subject. They made the points that research scans are typically not of the quality needed to make a clinical assessment and, second, obtaining a clinical scan is very expensive and can jeopardize future medical insurability.

One of the greatest sources of discussion among the group was whether or not a physician competent to read scans should be part of all research imaging studies when the principal investigator does not have those qualifications or is not trained as a medical doctor. The majority of the working group maintained that when a research protocol provides for communicating an incidental finding, the principal investigator is obligated to have the presence of a finding validated by a physician competent to read the scan. However, researchers in nonmedical settings may not have access to physician support.

Furthermore, communication itself is an issue when the principal investigator is not a physician trained to communicate medically sensitive information. This makes a single approach to the communication question elusive and should be the subject of further discussion and study.

Well-recognized ethics arguments in the medical screening (*13*) and genetics literature (*14*), for example, support a subject's right not to know. Investigators have an obligation to provide this option to subjects in the consent process. The subject opt-out option does expose investigators to a significant ethical conundrum, however, in the event of a clearly identified, life-threatening, treatable lesion. In that case, respecting subject autonomy is difficult to reconcile with a Good Samaritan ethos.

Although it is the investigator's choice to determine the scans necessary to meet the scientific goals of a study, it is the Institutional Review Board's (IRB's) responsibility to ensure that pathways for handling incidental findings in studies are explicit in the research protocol and in the written and verbal informed consent process. Investigator training should address explicit procedures for managing incidental findings. The pathway should address who will evaluate a suspected incidental finding and to whom the finding will be communicated. Statistics about the incidence of unexpected findings and the proportion with potential clinical significance may fruitfully be offered in the consent process; the sources of the data should be cited.

Investigators may worry that asking a physician to verify the presence of a suspicious incidental finding will compromise the subject's privacy. Researchers may seek consent for such communication in the process of obtaining the subject's consent to participating in the research. Alternatively, the researcher may de-identify the data before transmission. However, communication even with identifiers may well be allowed under state and federal privacy law because it is for the purpose of potential treatment. IRBs should recognize that some principal investigators might elect to opt out of evaluating incidental findings at all. This choice should be communicated to the IRB in protocols submitted for review and to research subjects during the process of obtaining informed consent.

Concerns about the sheer numbers of scans and the cost burden of routine readings were central to participants both affiliated with and not affiliated with medical centers. The group discussed how costs could be mitigated by discounts for research, for scan volume, or by written acknowledgment. Academic acknowledgment, as in publication, may be appropriate if a physician is a full member of the research team.

We saw no ethical requirement to acquire additional screening or clinical scans beyond those required for the research. Although we noted that intramural researchers at the NIH Clinical Center and investigators at some other institutions may obtain a clinical scan screened by a neuroradiologist for each subject in an imaging study, the majority of our group felt that requiring a clinical screening for each participant would be overly costly and impractical considering the unknown incidence of true-positive, clinically significant incidental findings in asymptomatic individuals. This is a consideration particularly for the growing number of research settings in which imaging studies are not performed within medical centers.

Our work lays the foundation for handling incidental findings in brain imaging, but further research is needed to evaluate the costs and benefits of identifying incidental findings and referring subjects for follow-up. How the burden of false-positives, combined with the burden of testing for incidental findings, weighs against the problem of missed incidental findings must be assessed. We must understand the downstream financial cost on the investigative process, and the psychological and financial burden that discovery of an incidental finding might have on subjects, in parallel with thinking about incidental findings upstream. The impact of an unexpected finding on a parent when a child is concerned, for example, is a particularly compelling problem. To this end, determining the frequency of confirmed and false-positive findings and developing age-appropriate and even disease-specific databases is imperative.

Beyond the points discussed here, we also concluded that it is premature to attempt to identify incidental findings in imaging data about brain function. This is the case whether the nonmorphologic brain data are obtained from MRI or other types of functional imaging. We recommend, however, that researchers remain aware of the issues surrounding incidental findings as single-subject functional data become better understood.

Other ethical challenges surfaced during the course of our work. These included, for example, responsibility and management of findings that occur in secondary data analysis through shared databases. Additional issues, such as those that may arise for third parties upon a finding suggestive of heritable disease, were also raised for future analysis.

Our recommendations, like our disagreements, were guided by our commitment to scientific and ethical responsibility. Legal considerations are important, but they did not drive this effort. Our desire is to be proactive in order to ensure scientific integrity and to engender public trust. Even while future research on this topic evolves, investigators must have a method in hand for grounding their reasoning and choices for handling incidental findings.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5762/783/DC1

CELL BIOLOGY

Double Knockout Blow for Caspases

Cells commit suicide with the assistance of factors released from mitochondria. Control of this process is now shown to involve feedback of later steps onto earlier ones.

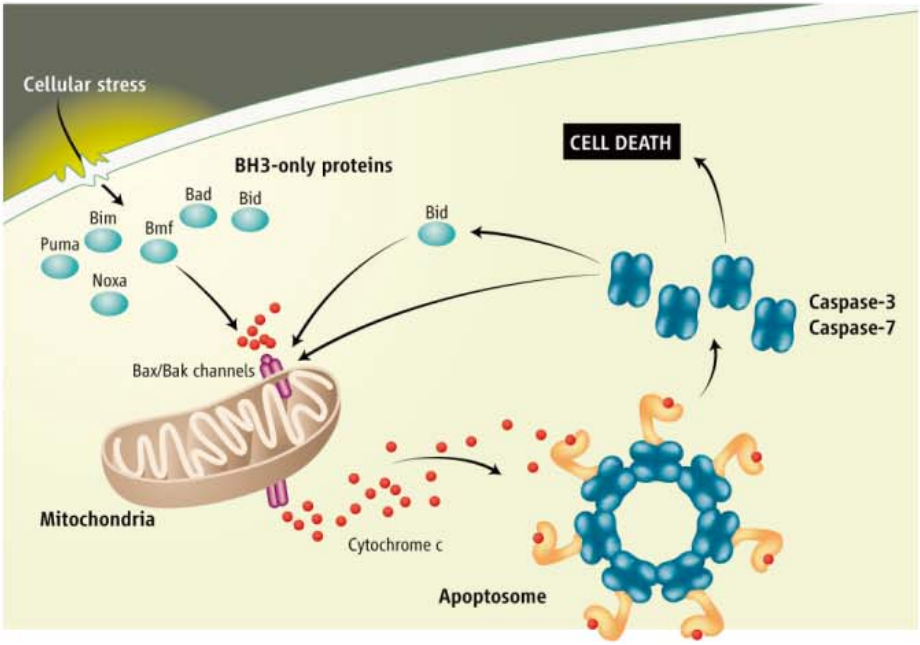
Colin Adrain and Seamus J. Martin

Eukaryotic cells can self-destruct in an orderly, highly controlled manner, and this is exploited to sculpt tissues during development or to eliminate infected, injured, or aged cells in the adult. This process, called apoptosis, is the best understood mode of regulated cell death, and involves the coordinate demolition of intracellular structures by members of the caspase family of proteases (*1*). Caspases are present as dormant enzymes in healthy cells but become unleashed during apoptosis to wreak controlled chaos, culminating in cell death. A major route to caspase activation and apoptosis results from cellular stresses—such as cytokine deprivation, heat stress, or DNA damage—that provoke permeabilization of the outer membrane of mitochondria, resulting in the release of cytochrome c and other factors into the cytosol. In the cytosol, the normally benign cytochrome c promotes the assembly of a caspase-activating complex, called the apoptosome. The apoptosome promotes activation of downstream caspases, thereby unleashing a torrent of protease activity within the cell. Because of these rather severe consequences, the mechanism of cytochrome c release from mitochondria has been intensively studied (*2*). A report by Lakhani *et al.* on page 847 of this issue (*3*), describing the phenotype of a double-knockout mouse lacking caspases 3 and 7, now provides evidence that the latter caspases may influence cytochrome c release and other mitochondrial events during apoptosis.

The "BH3-only" proteins are sentinels for cellular stress or damage in the mitochondrial (intrinsic) pathway to apoptosis (*4*). BH3-only proteins are activated in response to diverse triggers of apoptosis and initiate mitochondrial outer membrane permeabilization by acting as ligands for a mitochondrial membrane channel comprising the proteins Bax and/or Bak (*5*). Although the precise architecture of the Bax/Bak channel remains unclear, opening of this channel permits the escape of cytochrome c into the cytosol, where it triggers assembly of the apoptosome complex comprising Apaf-1 and caspase-9. In turn, the apoptosome activates caspases 3 and 7, setting off a chain of caspase activation events downstream (see the figure).

Mitochondrial cytochrome c release appears to be a defining event that marks the point of no return in the cell death pathway. Even if caspases are inhibited downstream of cytochrome c release, mitochondrial outer membrane permeabilization appears to be sufficient to kill most cells, probably because of a resulting decline in mitochondrial function. However, an important caveat is that cells dying without the participation of caspases do not display the hallmarks of apoptosis (typically extensive plasma membrane blebbing, DNA fragmentation, and the separation of the cell into intact fragments called "apoptotic bodies") and undergo a less neighborly form of cell death akin to necrosis. This has important implications, as it suggests that therapeutic intervention—for example, to prevent cells dying during stroke, myocardial infarction or hepatitis—downstream of cytochrome c release is likely to be futile.

A widely held view is that cytochrome c release is an all-or-none phenomenon in that all mitochondria within an individual cell either retain their cytochrome c or release all of it at the same time. It is also thought that downstream caspases do not influence this event. However, some observations are at odds with this view. Many extra cells survive in the brains of animals lacking Apaf-1, caspase-9, or caspase-3 (*6*–*8*), and these observations are difficult to reconcile with the view that mitochondrial outer membrane permeabilization is the point of no return on the road to death. There are several possible explanations for these observations, one of which is that caspase activation is required to amplify the initial damage caused either by low levels of stress or in particular cell types.



Routes to caspase activation in the intrinsic pathway to apoptosis. BH3-only proteins, activated in response to diverse forms of cellular stress, promote opening of Bax/Bak channels in mitochondria and release of cytochrome c. Cytochrome c activates the apoptosome, which in turn activates caspases 3 and 7 and other downstream caspases (not shown). Caspases may feed back directly on mitochondria and/or promote Bid activation to amplify cytochrome c release and trigger loss of mitochondrial transmembrane potential. The latter amplification loop may be critical for the death of certain cell types such as cardiomyocytes and neurons.

Lakhani *et al.* provide evidence that downstream caspases may amplify Bax translocation to mitochondria as well as cytochrome c release during apoptosis. The authors generated mice deficient in caspases 3 and 7 and found that, whereas mice deficient in either caspase were viable on this genetic background, those that lacked both caspases had defects in heart development and died immediately after birth. Assays using caspase-3– and caspase-7–deficient embryonic fibroblasts and thymocytes revealed substantial protection against diverse proapoptotic stimuli. These cells were also defective in several characteristic features of apoptosis.

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However, the most surprising defect seen in these embryonic fibroblasts was a pronounced delay in the kinetics of cytochrome c release and Bax translocation in response to ultraviolet radiation. This suggests that caspases 3 and 7 may participate in a feedback amplification loop to promote mitochondrial cytochrome c release in some circumstances. How might this operate?

It is already known that the BH3-only protein Bid can be activated through proteolysis by caspase-8 and caspase-3 (and possibly also by caspase-7). Thus, where only a subpopulation of mitochondria may have initially undergone mitochondrial outer membrane permeabilization, Bid proteolysis by downstream caspases may further enhance Bax activation to amplify cytochrome c release and seal the cell's fate (see the figure).

But is amplification of mitochondrial damage by caspases merely incidental, or can this dictate whether cells will live or die in some situations? In the case of postmitotic cells, such as neurons and cardiomyocytes, it seems that caspase activation may be essential for death. Exposure of the latter cell types to stimuli that induce mitochondrial outer membrane permeabilization under conditions where caspase activation is pharmacologically blocked resulted in survival of cells for days (*9*) and recovery upon restoration of normal culture conditions (*10*). This argues that certain cell types may be rescued from apoptosis by blocking this feedback amplification loop, and this may help to explain the failure of normal heart development in the caspase-3/caspase-7 double-knockout animals. Thus, postmitotic cells that have limited capacity to regenerate may not be discarded as readily as mitotic cells and may observe different death rituals as a result.

Although we now know much about how caspases are activated during apoptosis, it remains unclear why so many of them become engaged in dying cells and what they all do. Hundreds of mammalian caspase substrates have been identified to date, yet the consequences of only a handful of caspase-mediated proteolytic events are understood. British author W. Somerset Maugham once admonished that "Death is a very dull, dreary affair, and my advice to you is to have nothing whatsoever to do with it." We disagree.

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SOCIOLOGY

Experimental Macro Sociology: Predicting the Next Best Seller

A popular book, movie, or song can generate millions of dollars. But the social process that creates a blockbuster makes it difficult to predict which ones will succeed.

Peter Hedström

Sociologists typically are not concerned with explaining the behavior of single individuals. Their focus is instead on collective or social outcomes such as the strength of social norms like those of reciprocity and fairness; the size of social movements; the extent to which cities are racially segregated or workplaces are gender segregated; or various forms of inequalities like those in earnings and school performance. That is, the focus is on collective or macrolevel properties that are not definable for a single member of the collectivity. But to make sense of such properties, it is essential to take into account the actions and interactions of the individuals that brought them about (*1*). The study by Salganik *et al.* on page 854 in this issue (*2*) shows how large-scale Internet (World Wide Web)–based experiments can help us understand the complex processes that generate such outcomes.

With a little help from my friends. When making choices, individuals are influenced by what others think is best, making the final outcome unpredictable.

About 10 years ago, Coleman (*3*) pointed out that even if we are exclusively interested in explaining the relationship between two macro-level properties—for example, how patterns of social interaction influence the strength of certain norms—a proper explanation would require us to try to explicate the microlevel processes that brought it about. Thus, we must understand how macrolevel states at one point in time influence the individuals' orientations to their actions, preferences, beliefs, etc.; how these orientations to action influence how individuals act; and how these actions of individuals generate the macrolevel outcomes that we seek to explain.

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The transition from micro to macro has been the main intellectual hurdle for the development of sociological theory. The reason for this can be sought in the complexities involved. Whereas the other two links (macro to micro and micro to micro) can often be analyzed as if they concerned the actions of a single representative agent, once the micro-to-macro link is brought into the analysis, we are dealing with a dynamic process in which people react individually to an environment that consists mainly of other individuals who are reacting likewise.

Empirical sociological research usually is based on survey data—that is, on data derived from questionnaires addressed to random and representative samples of the population at large. Such data are excellent for many purposes, but they are not particularly useful for understanding or testing theories about interactive social processes. The data-collection design is generally such that one ends up with rich information about the attributes of individuals, but little information on the actions of those with whom these individuals interact. Although the statistical methods for analyzing contextual and social-interaction effects on the basis of observational data have

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developed considerably in recent years (*4*, *5*), so-called selection and omitted-variable bias make causal inference based on such data difficult indeed (*6*).

Salganik *et al*. (*2*) circumvent many of these problems by using experimental rather than observational data. They created a Web-based world where more than 14,000 individuals listened to previously unknown songs, rated them, and freely downloaded them if they so desired. Subjects were randomly assigned to different groups. Individuals in only some groups were informed about how many times others in their group had downloaded each song. The experiment assessed whether this social influence had any effects on the songs the individuals seemed to prefer.

As expected, the authors found that individuals' music preferences were altered when they were exposed to information about the preferences of others. Furthermore, and more importantly, they found that the extent of social influence had important consequences for the collective outcomes that emerged. The greater the social influence, the more unequal and unpredictable the collective outcomes became. Popular songs became more popular and unpopular songs became less popular when individuals influenced one another, and it became more difficult to predict which songs were to emerge as the most popular ones the more the individuals influenced one another.

It is important to note that these results refer to a situation in which individuals were randomly assigned to different groups and all individuals evaluated exactly the same set of alternatives. Nevertheless, when individuals influenced one another, identical populations that started from identical initial conditions each reached different final states. These findings are of considerable sociological importance. They offer persuasive evidence in support of one of the core ideas of sociology; namely, that the structure of social action—that is, the pattern and strength of social influence—in and of itself is of considerable importance for explaining the social phenomena we observe.

The Salganik *et al.* study also makes an important methodological contribution by showing how the Web can be used for conducting large-scale experiments. Experimental sociology typically is conducted in traditional lab settings and the focus is on small-group processes. Moving beyond the small group poses enormous logistic difficulties. To study multiple realizations of a collective social process requires thousands of participants. Experimental methods therefore have been thought to be of interest mainly to social psychologists. Salganik *et al*. show how the technology of the Internet can be used for overcoming these restrictions. They show that experimental macrosociology that takes into account all the three micro-macro relationships that Coleman discussed are indeed possible. They also demonstrate the importance of explicitly taking social influences into account when modeling micro-macro linkages. These are major contributions to the discipline at large.

As Salganik *et al*. show, social processes are highly path-dependent because what others have done in the past influence what we do in the present. Their study surely will influence others to use similar methodologies, but only time can tell whether this type of approach will be a "best seller" within the discipline. Social processes like these always are unpredictable.

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ASTRONOMY

Is the Mystery of Cosmic Magnetic Fields Solved?

Ruth Durrer

The origin of the magnetic fields seen throughout the cosmos has been puzzling. New calculations show that the density fluctuations in the early universe can produce fields of the right amplitude.

Observing astrophysical magnetic fields is difficult. Nonetheless, fields of surprisingly consistent amplitudes on the order of microgauss have been discovered in many galaxies and clusters of galaxies (*1*). So far, the generation of these fields has remained a mystery. For a long time, scientists tried to conceive of a mechanism by which tiny primordial fields would be created in the early universe (*2*). Later, during gravitational collapse, such fields could be amplified—for example, by means of a dynamo mechanism—and thereby lead to the observed fields in galaxies and clusters. Even if the seed fields needed for dynamo amplification were as small as 10^{-25} G or smaller, these primordial seed fields have been shown to be severely constrained by the gravity wave background that they induce (*3*, *4*). As Ichiki *et al*. report on page 827 of this issue (*5*), there is another possibility. They show that second-order cosmological perturbations necessarily generate magnetic fields that are of the right order to be amplified by the dynamo mechanism into the currently observed fields in galaxies and clusters.

This is a very exciting proposal. It implies that tiny magnetic fields on the order of 10^{-22} G are present even in intergalactic space (see the figure). Furthermore, the clustering properties of magnetic fields carry an imprint of the primordial fluctuation spectrum from inflation (*6*). If true, magnetic fields might come to play a very important role in cosmology, comparable to the cosmic microwave background (CMB) anisotropies. In analyzing these fields, we might learn about the physics of inflation that occurs at very high energies, probably about 10 orders of magnitude higher than the energy that will be reached at the Large Hadron Collider, the world's largest particle accelerator now under construction at CERN in Geneva.

During ordinary inflation, a period where the temperature may reach $T \approx 10^{14}$ GeV (*7*), no magnetic fields are generated, because electromagnetism cannot be induced by the expansion of the universe. Only when introducing additional quantum fields can one generate magnetic fields during inflation (*2*). On the other hand, at the electroweak phase transition, which takes place at a temperature of $T_{ew} \sim 200$ GeV, where the electromagnetic and weak nuclear forces separate, magnetic fields can be generated and their amplitude might be correlated to the baryon number produced during the transition. Within the standard model, both mechanisms, the production of baryons and of magnetic fields, are too inefficient to explain the observations, but in slightly generalized (e.g., supersymmetric) versions, the production of sufficient seed fields might be possible (*2*).

The most problematic aspect of such primordial scenarios is, however, that the galactic scales we are interested in are far larger than the Hubble scale (*8*) at the time of magnetic field generation. This leads to very substantial generation of gravitational waves during horizon crossing (*9*). This is in conflict with the helium abundance in the universe, which requires that at the time of nucleosynthesis, when $T \sim 0.1$ MeV, the gravity wave contribution to the energy density of the universe must have been less than about 10%.

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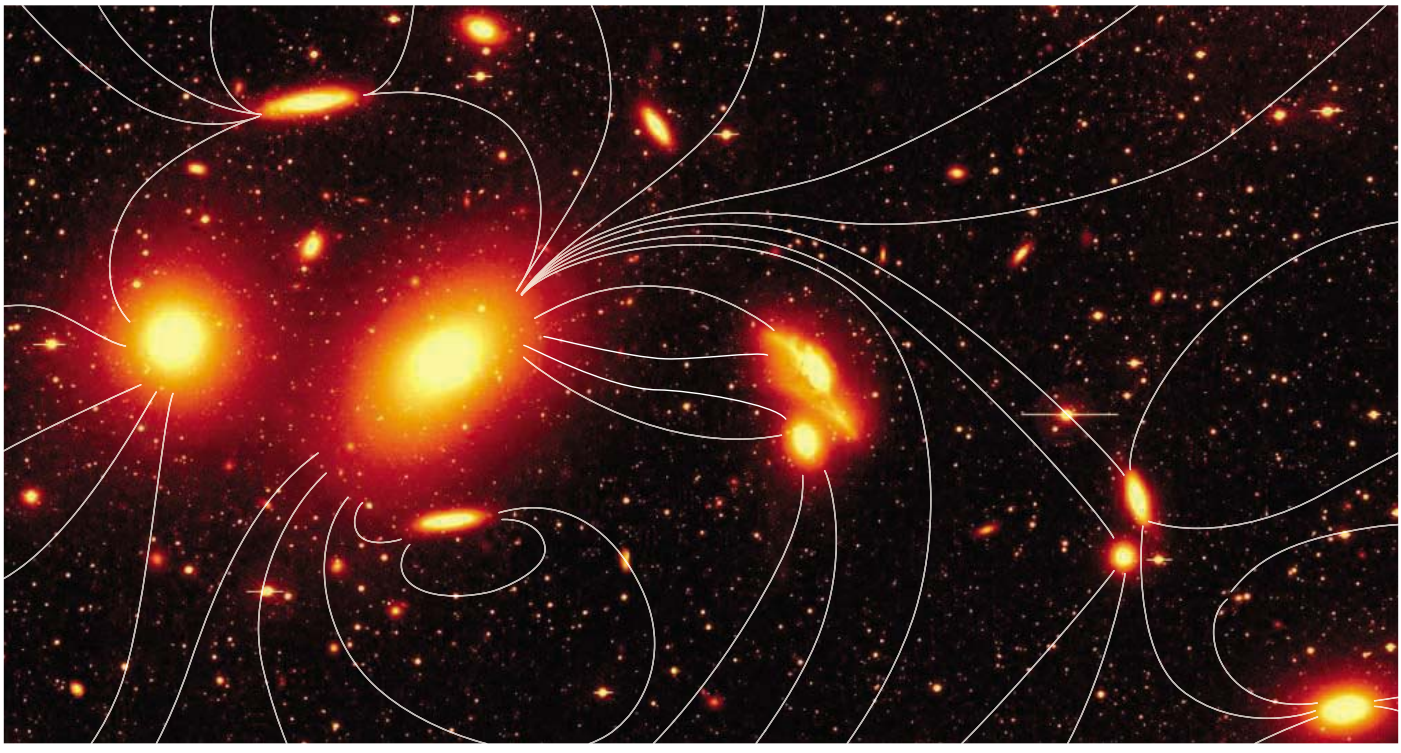

Intergalactic magnetism. The Virgo cluster with one possible configuration of magnetic field lines (artist's impression). [Original Virgo image from (*15*)]

This limit is severely violated by nearly all of the proposed models, because the gravity wave energy density usually has a very blue spectrum (*10*). In order to obtain sufficient magnetic field amplitudes on large scales, the models violate the constraints required by nucleosynthesis on small scales (*3*, *4*).

The problem mentioned above is entirely absent if magnetic fields are generated by second-order effects because they become relevant much later, around recombination at $T_{rec} \sim 3000$ K ~ 0.3 eV. Furthermore, second-order effects are certainly present, so that these magnetic fields will be produced under all circumstances.

To first order in perturbation theory, the velocity fields of electrons and protons, which form a perfectly coupled fluid, are equal, and hence no currents and vorticity are generated (*11*, *12*). Furthermore, the magnetic field does not couple to the background geometry. Currents are generated and geometric effects come in only if second-order effects are included. In previous papers, isolated effects such as the amplification of magnetic fields by gravity waves (*13*) and the amplification of gravity waves by the magnetic field energy (*3*, *4*) have been studied. But it was always clear that these effects are not the only ones and that they interact with each other and with other second-order effects. However, the fully relativistic general second-order equations for the Einstein-Maxwell system in cosmology and a systematic discussion of all the possible effects are still missing.

Ichiki *et al*. argue (*5*, *14*) that magnetic fields are produced mainly by the difference in the electron and photon velocities and by the coupling of the electron velocity to photon anisotropic stresses. They compute these magnetic fields and obtain $B_{rec} \sim 10^{-16.5}$ G after recombination. Subsequently, the number of magnetic flux lines passing through a given surface remains constant. Hence, if distances in the universe expand by a factor $(z+1)$, where z is the redshift, the magnetic field is reduced by a factor $1/(z+1)^2$. With $z_{rec} \sim 1000$, this gives $B_0 \sim 10^{-22.5}$ G, where the index 0 indicates the present value. When the plasma collapses into galaxies, electrons and protons drag the magnetic flux lines with them and enhance the magnetic fields by an additional factor of $B_{gal} \approx (\rho_{gal}/\rho_0)^{2/3} B_0 \approx 10^3 B_0$, where ρ_{gal} is the mass density in a galaxy and ρ_0 is the present mean density. Subsequent amplification by the dynamo effect could easily lead to the observed magnetic fields in galaxies.

In their report in this issue, Ichiki *et al*. (*5*) argue that second-order perturbation can induce magnetic fields of the right amplitude for the observed fields in galaxies and clusters. This idea—which is certainly correct, at least in principle—is beautiful because it ties together cosmic magnetic fields with CMB anisotropies, weak lensing, and cosmic large-scale structure. If the effects proposed by Ichiki *et al.* are really the dominant ones, and if they are as large as those obtained here (this is still a matter of debate), the magnetic seed field spectrum can be calculated easily and may lead to a new cosmological observable. This prospect is exciting, but detailed calculations are now required to check that no additional important second-order effect has been overlooked.

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6. Inflation is a process thought to have taken place in the very early universe. It has rendered the universe large and homogeneous and has generated the small fluctuations that are observed in the CMB.
7. The temperature of the universe after inflation is not known. This is simply a typical value. We often express temperature in GeV: 1 GeV = 10^9 eV ~ 10^{13} K.
8. The Hubble scale at a given time t of the expanding universe is the distance a photon has traveled since the Big Bang until time t. The present Hubble scale is about 1.3 × 10^{10} light-years.
9. During the expansion of the universe, the Hubble scale grows faster than the physical size of a perturbation. The instant when the latter becomes smaller than the Hubble scale is called horizon crossing. By causality, only after horizon crossing can a perturbation "notice" that it is in fact a perturbation and begin to oscillate, e.g., like a gravitational wave.
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CELL SIGNALING

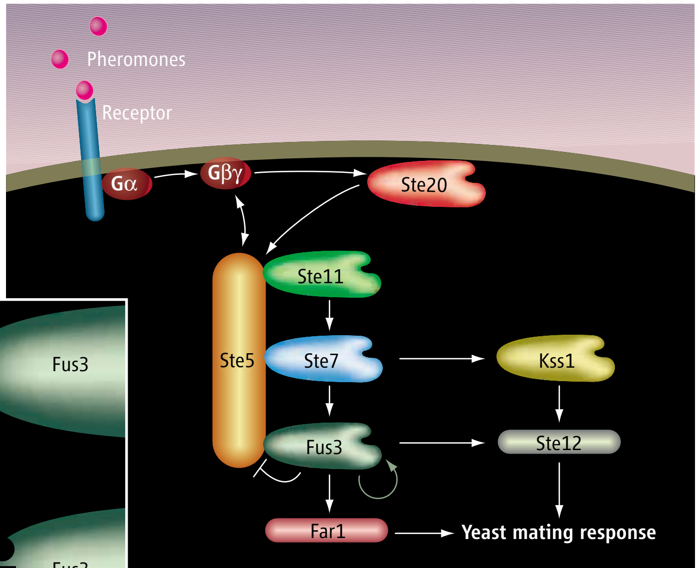
A Sophisticated Scaffold Wields a New Trick

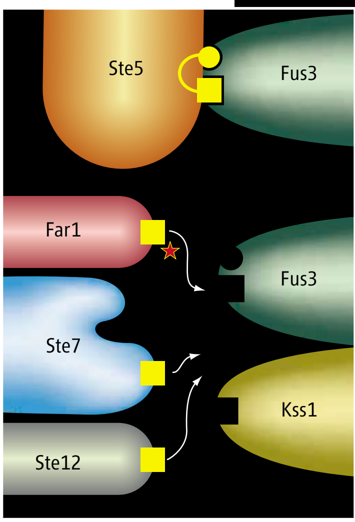
Many cellular processes are governed by proteins that are docked onto a scaffold protein. In addition to providing binding sites, scaffolds influence the signaling output of these multiprotein complexes.

Ashton Breitkreutz and Mike Tyers

A complex and dynamic network of signaling proteins enables eukaryotic cells to respond to different stimuli, including changes in their physical environment, contacts with other cells, and growth and differentiation factors. Mitogen-activated protein kinase (MAPK) modules often help accomplish this task. (*1*). The archetypal MAPK signaling cascade mediates the mating response of budding yeast (see the figure). In response to pheromone, upstream events activate the MAPK kinase kinase Ste11, which then phosphorylates and activates the MAPK kinase Ste7, which in turn phosphorylates and activates two partially redundant MAPKs, Fus3 and Kss1 (*2*). MAPK activation requires dual phosphorylation on a threonine (T) and a tyrosine (Y) in a conserved T-X-Y (X, any amino acid) motif in the kinase activation loop. In addition to the tiered activation steps in the MAPK cascade, the pheromone pathway kinases are functionally connected by the scaffold protein Ste5 (*3*). Metazoan MAPK scaffold proteins, such as KSR (kinase suppressor of Ras) and JIP (c-Jun N-terminal kinase inhibitory protein), have also been discovered (*1*). As befits their name, scaffolds were originally thought to act as passive docking sites, functioning to localize and concentrate the appropriate components for signal transmission (*3*, *4*). However, at least two lines of evidence hint that Ste5 might play a more active role in signal transmission. First, in vitro studies that reconstituted the yeast pheromone–responsive MAPK cascade suggest that Fus3 is activated in a distinctly stepwise fashion, with highest activity achieved in the presence of Ste5 (*5*). Second, synthetically tethering the kinases to Ste5 with ectopic protein interaction domains only partially rescues the mating defect of *ste5* mutants (*6*). On page 822 of this issue, Bhattacharyya *et al.* (*7*) demonstrate that Ste5 indeed plays an active role in signaling, and a surprisingly dynamic one at that.

In the first glimpse of a scaffold bound to a MAPK, Bhattacharyya *et al.* crystallized a 29-residue polypeptide of Ste5 in complex with Fus3. The Ste5 peptide extends over the back side of Fus3 and engages one site on the N-terminal lobe of the kinase (site A) and another on the C-terminal lobe (site B). An intervening disordered region of eight residues links the two MAPK-binding regions of the Ste5 peptide. At the A site, Ste5 interdigitates into the normal five-stranded β-sheet structure of the N-terminal lobe to create a seven-stranded β-sandwich architecture. The B site precisely overlaps with a previously described MAPK surface groove that binds to docking peptides present in a variety of substrates, as well as in the upstream MAPK kinase (*8*, *9*). Unexpectedly, the Ste5 peptide allosterically induces autophosphorylation of the tyrosine in the T-X-Y activation loop of Fus3, which leads to a substantial degree of kinase activation (*7*). This autoactivation event is intramolecular in nature and requires an optimal length linker between the two MAPK-binding regions in the Ste5 peptide. Other peptides known to interact with Fus3 did not have this effect. Ste5 is also phosphorylated on a threonine residue by autoactivated Fus3. Mutation of this Ste5 modification site increases the expression of a mating-responsive gene, implying that the Ste5-MAPK module attenuates its own activity (*7*). All told, these findings dramatically elaborate the repertoire of Ste5 functions.





The mating pathway in budding yeast. In response to ligand-induced activation of a pheromone receptor, activated Gβγ protein subunits in the membrane recruit and oligomerize Ste5, which in conjunction with the PAK-like kinase Ste20, activates the MAPK module. Once activated, the MAPKs Fus3 and Kss1 phosphorylate a variety of proteins that effect the pheromone response, including the transcription factor Ste12 and the polarization factor and cyclin-dependent kinase inhibitor Far1. Not all signaling components or substrates are shown [see (*2*) for details]. **(Inset)** Circle indicates peptide motif from Ste5 that docks specifically into the A site of Fus3. Squares indicate the peptide motifs from Ste5, the MAPK kinase Ste7, and various substrates that dock into the B sites of both Fus3 and Kss1; the star on Far1 indicates that it is a Fus3-specific substrate (*9*).

As with any breakthrough, a host of new questions arise. How the presumed tension exerted by the Ste5 linker on the two lobes of Fus3 facilitates autophosphorylation is not clear. However, this type of mechanism is not entirely without precedent. For example, modulation of the N-terminal lobe through peptide interactions is used to activate other kinases, such as protein kinase A or Akt/protein kinase B (*10*, *11*). In another sense, Ste5 is loosely analogous to the protein cyclin, which binds and activates the cyclin-dependent kinases that control the cell division cycle. (*12*). And, as Bhattacharyya *et al.* note, the activation of Fus3 by Ste5 is reminiscent of the autoactivation of the mammalian MAPK p38α by its binding partner, TAB1 (*13*). Finally, intramolecular autophosphorylation has also been docu-

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mented as an intrinsic maturation step for the dual-specificity tyrosine phosphorylation–regulated protein kinases that are distantly related to MAPKs (*14*). The fact that Fus3 phosphorylates itself on tyrosine belies its categorization as a serine-threonine kinase, and begs the question as to whether Fus3 may phosphorylate other substrates on tyrosine residues.

Given the overlapping binding sites on Fus3, competition for binding partners both within the Ste5-MAPK complex and for other Fus3 substrates must be rife, especially because many of the peptide interactions occur with moderate to weak affinity (*7*, *9*). Fus3 does not detectably interact with either the A- or the B-site fragment of Ste5 alone, in agreement with the finding that the Ste5-Fus3 and Ste7-Fus3 interactions are competitive (*15*). This competition implies that a dynamic series of events occurs during signal transmission. Then there is the issue of Kss1, which is also activated in the pheromone response (*5*, *16*). Kss1 does not, however, interact with the Ste5 fragment that binds Fus3, and instead requires Ste7 for recruitment to Ste5 (*5*). Because Kss1 is still activated by pheromone in the presence of a catalytically inactive form of Fus3 (*5*, *17*), it seems plausible that both Fus3 and Kss1 are found together in oligomerized Ste5 complexes, which are necessary for signaling (*18*).

From the signal transmission perspective, yet other issues come to the fore. If Ste5 triggers partial activation of Fus3 by autocatalytic tyrosine phosphorylation, what then is the role of Ste7? Through mutational analysis, Bhattacharyya *et al*. clearly demonstrate that the pheromone response requires Ste7-mediated dual phosphorylation of Fus3 and not Ste5-mediated monophosphorylation (*7*). Indeed, it is unknown whether Fus3 is ever monophosphorylated on tyrosine in vivo. If this form of Fus3 does exist, what role might it play in modulating the signaling response? Another canonical MAPK module, the p42/44 Erk1/2 pathway that triggers maturation of frog (*Xenopus laevis*) oocytes, displays a profound all-or-none or "switchlike" response to stimulus (*19*). In yeast, however, careful single-cell analysis has recently demonstrated that yeast respond gradually to increasing doses of pheromone in a graded rather than switchlike response (*20*). By potentially eliminating the distributive phosphorylation of Fus3, Ste5 might flatten the dose-response curve of Fus3 activation, as would the observed feedback inhibition of Ste5.

Unlike the conserved enzymatic components of signaling networks, scaffolds are not easily recognized by sequence similarity alone and so have undoubtedly been understudied to date. The rather dull moniker "scaffold" has also perhaps not helped the profile of these interesting proteins. Scaffolds have been discovered not only as components of MAPK modules, but also in many other signaling pathways, such as the ubiquitous A-kinase anchoring proteins (AKAPs) that confer spatial specificity on protein kinase A activation (*21*). Spurred on by the ground-breaking studies of Bhattacharyya *et al.*, odds are that more scaffold magic will soon be discovered.

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CHEMISTRY

Building Molecules with Carbon Monoxide Reductive Coupling

Brad Wayland and Xuefeng Fu

A new reaction can generate complex organic molecules from carbon monoxide. This method for creating organic materials could provide fuel and chemicals that do not depend on our dwindling petroleum supply.

Uncertainty in petroleum cost and supply demands that alternative sources of primary organic materials be developed (*1*). Carbon monoxide as a component of synthesis gas (nH_2 + CO) from coal and biomass will have a leading role in meeting these needs (*2*). One prominent objective of energy-related research is to develop strategies that use CO as a two-carbon or larger building block to construct the organic materials needed for fuels and chemical manufacturing. Transition metal catalysts are highly effective in directing reactions of carbon monoxide as a one-carbon building block, but catalysts that promote C–C coupling of CO have not yet been developed. Determining the scope and nature of carbon monoxide reactions with metal complexes that result in C–C bond formation provides guidelines for the design of catalysts for CO coupling reactions. An important new type of CO coupling is reported on page 829 of this issue by Summerscales *et al*. (*3*) in which a set of three CO molecular units combine to form an oxocarbon dianion ring, $C_3O_3^{2-}$, by means of an organouranium complex.

Enhanced online at www.sciencemag.org/cgi/content/full/311/5762/790

Observation of the actinide complex containing the elusive $C_3O_3^{2-}$ unit both encourages a renewed exploration for additional classes of metal-induced CO reductive coupling reactions and advances the historic search for carbon monoxide reactions that directly produce the three-carbon member of the oxocarbon dianion series ($C_nO_n^{2-}$; $n = 3$ to 6). This quest began in the very earliest chapter of organic chemistry when in 1834 Liebig (*4*) reported that CO reacts with molten potassium to produce salts of the croconate ($C_5O_5^{2-}$) and rhodizonate ($C_6O_6^{2-}$) dianions. These five- and six-membered oxocarbon ring compounds had been reported to be formed from high-temperature reactions of KOH and carbon by Gmelin (*5*) in 1825. Croconic and rhodizonic acids are now known to be products of biological oxidations, and thus in hindsight Gmelin could be credited with the earliest organic synthesis from nonorganic substances. More than a century passed before the

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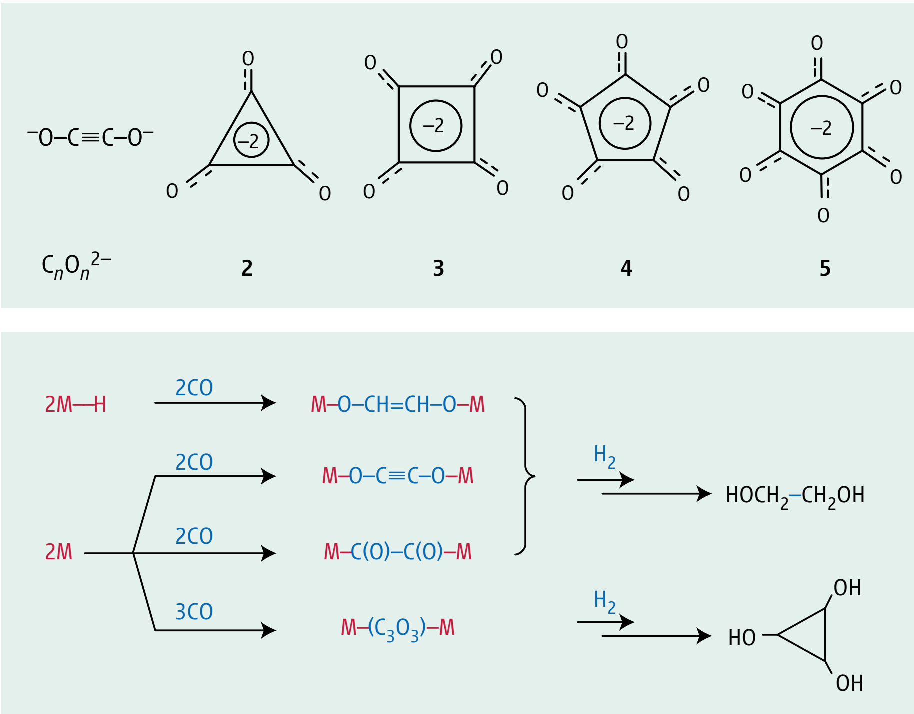
$C_nO_n^{2-}$ series was extended to the four-carbon squarate dianion by preparation of $C_4O_4^{2-}$ from the electrolysis of CO in polar solvents like dimethylformamide (*6*). Species containing the deltate dianion $C_3O_3^{2-}$ were prepared by directed synthesis from squarate (*7*), but eluded preparation from CO before the discovery of the uranium (III) reaction. The recurring fascination with this series of oxocarbon dianions ($C_nO_n^{2-}$; $n = 2$ to 6) (*8*, *9*) has alternately been stimulated by the simplicity and aesthetics of their symmetry (see the figure, top panel), issues of electronic structure and aromaticity (*10*), and currently because of renewed interest in the potential applications in energy-relevant molecular transformations.

Direct dimerization of CO to O=C=C=O is thermodynamically highly unfavorable (the Gibbs free energy $\Delta G°$ at 298 K is about +73 kcal mol^{-1}), and all of the neutral oxocarbon CO oligomers (C_nO_n) are unstable relative to dissociation into CO (*11*). Insertion of CO into an organo-metal bond (M–R) to form an acyl complex [M–C(O)R] is often a favorable process, but double insertions that place –C(O)– units in adjacent positions are generally thermodynamically unfavorable ($\Delta G°$ at 298 K ~ +10 kcal mol^{-1}) primarily from the increase in entropy for the addition of each CO unit ($T\Delta S°$ at 298 K ~ +8 kcal mol^{-1}). Productive CO coupling must occur in combination with another process that has an energy term that makes the overall reaction thermodynamically favorable. Formation of neutral (C_nO_n) species from CO is invariably thermodynamically unfavorable, but combining CO coupling with two-electron reduction produces stable oxocarbon dianion species $C_nO_n^{2-}$ including the two-carbon ethyne diolate ($^-$O–C≡C–O$^-$).

A prior "oil crisis" stimulated a fruitful burst of academic and corporate research on exploring strategies to obtain a variety of forms of CO coupling reactions and evaluating the potential for commercial processes. Several types of carbon monoxide reductive coupling were observed to be promoted by low–oxidation state oxophilic lanthanide (*12*), actinide (*13*), and early transition metal complexes (*14*) that form strong bonds with oxygen.



Carbon monoxide combinations. (Top) Structures of $C_nO_n^{2-}$ units for n = 2 to 6. **(Bottom)** Representative pathways for CO reductive coupling and hydrogenation.

Reactions of metal hydrides with CO that give formyl coupling to ethene diolates (M–O–CH=CH–O–M) is a particularly promising reductive coupling process associated with oxophilic metal complexes (*14*). Transition metal complexes were also devised to function as a template to organize the coupling and reduction of CO to an ethyne diolate unit stabilized by metal binding (*15*). Late transition metal complexes that prefer metal-carbon bonding were identified to form ethane dionyl complexes [(L)Rh–C(O)–C(O)–Rh(L)] that use the relief of steric effects as the additional driving force (*16*, *17*).

The pivotal objective in this area is to combine carbon monoxide coupling with hydrogenation to produce thermodynamically favorable organic products such as ethylene glycol (HOCH$_2$–CH$_2$OH) ($\Delta G°$ at 298 K ~ –7.2 kcal mol^{-1}). Realization of this objective relies on identifying catalysts that guide both the CO coupling reactions and product-forming steps (see the figure, bottom panel).

The remarkable reaction of CO with the uranium (III) complex reported by Summerscales *et al.* (*3*) illustrates how two metal sites can function in concert to assemble carbon monoxides into a three-carbon unit ($C_3O_3^{2-}$). Reductive coupled C–C bonded units provide a source of building blocks for constructing more complex organic structures. The types of metal complexes that were selected to illustrate the structures that result from CO reductive coupling have large thermodynamic or kinetic stability that often permit full structural characterization by x-ray crystal studies, but prohibit the product-forming steps and catalytic function. The time is past due to revisit CO reductive coupling from the focused perspective of achieving catalytic substrate reactions. Design of catalysts for CO reductive coupling processes must focus on circumventing the thermodynamic and kinetic restrictions by selecting ligand arrays that weaken oxophilic metal-substrate oxygen bonds and open reaction pathways with low activation energies.

The profound influence that the ligands have on the thermodynamic and kinetic factors for organo-metallic reactions provides confidence that a focused effort can produce effective catalyst materials. Carbon monoxide reductive coupling processes have the potential to contribute toward establishing a reliable supply of economical organic materials for both chemical manufacturing and liquid fuels. The prospects for identifying and evaluating the catalysts for timely application are dependent on the collective vicissitudes of research, economics, and politics.

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GE PRIZE WINNER

How Molecular Motors Move

Ahmet Yildiz

Myosin and kinesin motor proteins use the energy obtained from adenosine triphosphate (ATP) hydrolysis to transport organelles and vesicles by moving along the cytoskeleton. Structurally, these motors are dimeric, having two motor heads, two legs, and a common stalk. The head regions bind to actin or microtubule filaments and power the forward movement. The central question was how the two heads are coupled so that the motor can processively move along its track. In the hand-over-hand model (*1*), ATP binding and hydrolysis creates a conformational change in the forward head (head 1) and this conformation pulls the rear head (head 2) forward, while head 1 stays fixed on the track. In the next step, head 2 stays fixed and pulls head 1 forward. Alternatively, in the inchworm model (*2*) only the forward head catalyzes ATP and always leads while the other head follows (see figure below).

In both of these mechanisms, the motor needs two heads to be able to stay on the track as it moves and its step size depends on the length of the legs. However, myosin VI with short legs (8 nm) was observed to take the same long steps (30 nm) as myosin V. Moreover, a single-headed processive motor has suggested that two heads are not necessary for processive motion. These observations lead to another mechanism: biased diffusion of the motor along the actin/microtubule lattice (*3*). The bias is provided by the initial push of the power stroke, and the motor most likely attaches to the next binding site in the forward direction. Understanding motor protein movement is a fundamental step in understanding how cargo transport works within a cell, but despite intensive research, the mechanism underlying movement remained highly controversial.

The most direct way to distinguish among these models is to measure how much each head moves when the motor walks. The hand-over-hand model predicts that a head alternately moves twice the stalk displacement and stays stationary in the next step while the other head takes a step (see figure, left panel). In contrast, the inchworm model predicts that both of the heads move forward the same distance as the stalk (see figure, right panel). The diffusion model states that heads randomly bind to the track. Current nanometer-precision tracking techniques (optical traps and cantilever probes) cannot readily be used to watch the head movement, because they use a large probe (>100 μm) that might hinder the movement of the motor's tiny heads (5 to 10 nm). What is needed is to track a nanometer-sized probe (such as organic dyes) attached to a motor head with single- nanometer precision.

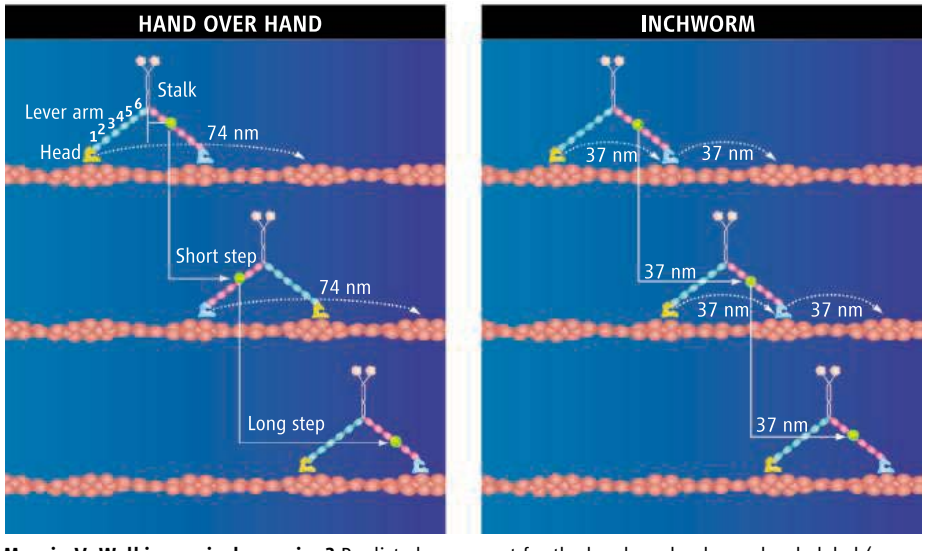
The position of a diffraction-limited spot can be localized very precisely by determining the center of its emission pattern. However, organic dyes are not very bright and the signal disappears quickly by permanent photobleaching. This limited previous single-molecule tracking experiments to a precision of around 30 nm (*4*). I have extended the photostability and brightness of single organic dyes 20 times by effectively deoxygenating the assay solution and using reducing agents, and I have achieved 1.5-nm localization and collected 1.4 million photons from single organic dyes. The technique, named fluorescence imaging with one-nanometer accuracy (FIONA), has improved spatial resolution in single molecule fluorescence by ~20-fold.

Using FIONA, I tracked the movement of the motor proteins myosin V, kinesin, and myosin VI, which were labeled with a single dye in the head region as follows.

Myosin V. Bifunctional rhodamine (Br)–labeled calmodulins were exchanged into the myosin V lever arm, where the calmodulin can potentially exchange at any of six calmodulin-binding sites (IQ domains). The inchworm model predicts a uniform step size of 37 nm regardless of the position of the labeled calmodulin. The hand-over-hand model predicts alternating short and long steps, depending on the in-plane distance of the dye from the midpoint of the myosin. The trajectory of moving spots created three classes of steps. I observed 74-or 0-nm displacements for dye on the first IQ domain, alternating 52-and 23-nm steps for dye on the fifth IQ domain and alternating 42-and 33-nm steps for dye on the sixth IQ domain (*5*) (see figure below, left).

Kinesin. A human kinesin was specifically labeled on the head region with a single Cy3 molecule. As the stalk took 8-nm steps, the head was observed to take alternating 16-nm and 0-nm steps (*6*).

Myosin VI. Myosin VI was labeled with a single Cy3 molecule on a calmodulin-binding site. Again, the labeled head alternately moved twice as far as the stalk moved and stopped as the other head moved (*7*). Unexpectedly, Cy3-calmodulin showed significant flexibility when it had ATP bound, whereas it was immobile in



Myosin V: Walking or inchworming? Predicted movement for the heads and a dye molecule label (green dot) on the lever arm in the hand-over-hand model (**left**) and the inchworm model (**right**). The FIONA assay has revealed that myosin V, along with kinesin, and myosin VI, walks hand-over-hand.

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YOUNG SCIENTIST AWARD

GE Healthcare and *Science*/AAAS are pleased to present the prize-winning essay by Ahmet Yildiz, a regional winner for North America who is the Grand Prize winner of the Young Scientist Award.

the nucleotide-free and ADP states. This implies that in some part of myosin VI's ATPase cycle, the lever arm uncouples from the motor, which could arise from elongation of the lever arm. Lever arm elongation may provide the long step (30 nm) of myosin VI with a short lever arm (8 nm).

Thus we have established a new, single-molecule fluorescence technique, FIONA, which is able to resolve steps of a few nanometers taken by molecular motors. FIONA assays on myosin V, myosin VI, and kinesin have revealed that these motors move by walking hand over hand, not by "sliding" like an inchworm, nor by "diffusing" along the cytoskeleton. FIONA is also a broadly applicable technique in other fields of molecular biology, such as DNA sequencing and particle tracking in vivo.

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2005 Grand Prize Winner >>

Ahmet Yildiz, the author of the prize-winning essay and a North American regional winner, grew up in Sakarya, Turkey. In 2001, he received a bachelor's degree in physics from Bogazici University, Istanbul, and started his graduate studies in biophysics at the University of Illinois Urbana-Champaign. Working in the research group of Dr. Paul Selvin, he developed the technique of fluorescence imaging with one-nanometer accuracy (FIONA). This work was recognized with a Foresight Institute Distinguished Student Award in 2003. He went on to use FIONA to study the molecular walking mechanism of the motor proteins myosin V, myosin VI, and kinesin. Dr. Yildiz received his Ph.D. in 2004, and his thesis was awarded the Gregorio Weber International Prize in Biological Fluorescence. In 2005, he moved to the University of California, San Francisco, where he is a postdoctoral fellow in the research laboratory of Dr. Ronald Vale. He is currently studying the structural mechanism of cytoplasmic dynein.

Regional Winners

North America: Nieng Yan for her essay, "Mechanisms of Programmed Cell Death in *Caenorhabditis elegans*." Dr. Yan was born in Jinan, China, in 1977 and grew up in Beijing. As an undergraduate at Tsinghua University, she developed a strong interest in science and was also deeply influenced by Beijing's unique civil milieu. After receiving a bachelor's degree in biology in 2000, she traveled to New Jersey to pursue graduate training in the Department of Molecular Biology at Princeton University. Under the guidance of Dr. Yigong Shi, she used structural biology and biochemistry techniques to elucidate the molecular mechanisms of cell death regulation. Dr. Yan received her Ph.D. in December 2004 and is currently completing research projects in Dr. Shi's lab. Her goal is to continue in an academic career.

Europe: Marina Aspholm for her essay, "Adaptation of *Helicobacter pylori* Adherence Properties in Promotion of Host Tropism and Inflammatory Disease." Dr. Aspholm comes from Kiruna, Sweden, a city famous for an ice hotel that is constructed anew each winter. Dr. Aspholm studied chemistry and molecular biology at Umeå University and received a Master of Science degree in 1998. She remained at Umeå University for Ph.D. studies through a fellowship from the Swedish Foundation for Strategic Research. Under the guidance of Dr. Thomas Borén, she examined how the gastric pathogen *Helicobacter pylori* adapts its adherence properties to fit predominant patterns of gastric mucosal cell surface glycosylation. During this time, she also collaborated with the group of Dr. Douglas Berg, Washington University, St. Louis, Missouri. Dr. Aspholm defended her Ph.D. thesis in 2004 and now holds an EMBO long-term fellowship and is a research scientist in the laboratory of Dr. Michael Koomey at the University of Oslo, Norway.

Japan: Rikinari Hanayama for his essay, "Impaired Phagocytosis of Apoptotic Cells and Development of Autoimmune Diseases." Dr. Hanayama was born in 1974 and grew up in Osaka, Japan. He obtained an M.D. degree from Osaka University in 1999. After a year as a medical intern, he decided to pursue basic research and joined the laboratory of Dr. Shigekazu Nagata as a graduate student. There he identified a molecule that promotes the phagocytosis of apoptotic cells and showed that the inefficient removal of the apoptotic cells can lead to autoimmune diseases. Dr. Hanayama was awarded a predoctoral fellowship from the Japan Society for the Promotion of Science in 2002 and received his Ph.D. and the Yamamura Award from Osaka University in 2004. After working as an instructor in genetics with Dr. Nagata, he joined the laboratory of Dr. Michael E. Greenberg at Children's Hospital Boston/Harvard Medical School with a long-term postdoctoral fellowship from the Human Frontier Science Program.

All Other Countries: Jianmin Zhang for his essay, "Establishment of Transcriptional Competence in Early and Late S Phase." Dr. Zhang was born in Tianjin, People's Republic of China. After graduating from Tianjin Medical University, he worked as a research associate at Tianjin Infectious Diseases Hospital. In 1996, he began graduate studies at the Hebrew University of Jerusalem, where he first obtained an M.Sc. under the guidance of Prof. Hagai Ginsburg in the Department of Biological Chemistry and then joined Dr. Howard Cedar's lab at the Hadassah Medical School. Life in a foreign country was made easier by the support he received from Dr. Cedar. His studies on gene repression suggested a mechanistic connection between DNA replication timing and gene expression. Dr. Zhang received his Ph.D. in 2004 and was awarded The Aharon Katzir Prize. He is now a postdoctoral fellow in Dr. Daniel Haber's laboratory at the Cancer Center, Massachusetts General Hospital and Harvard Medical School. Dr. Zhang's life was recently made richer by the arrival of a baby daughter.

For the full text of essays by the regional winners and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/prizes/ge/index.dtl

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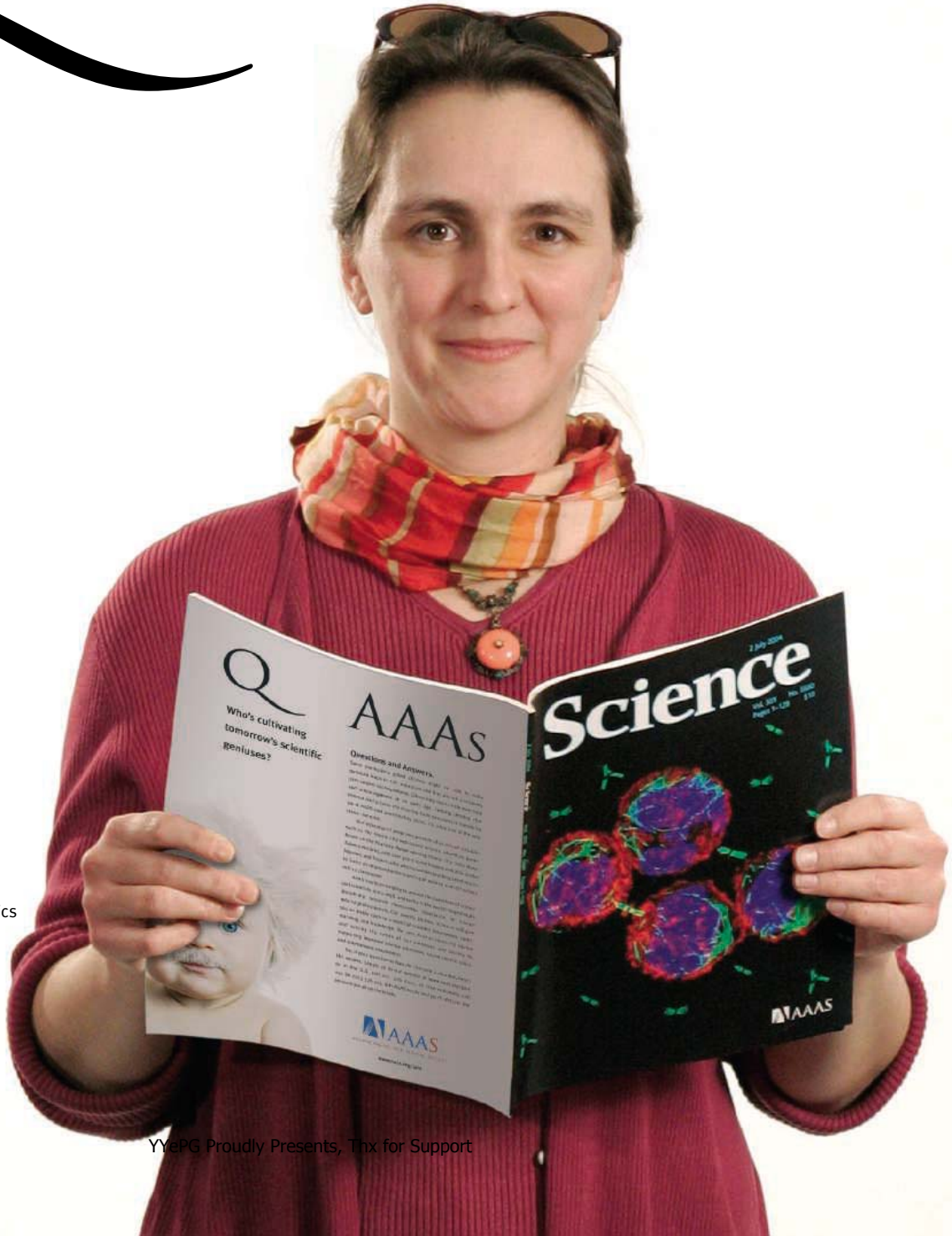
Who inspires brainwaves while I study water waves?



Katherine Socha Ph.D.
Assistant Professor of Mathematics
and AAAS member

AAAs

“I study the mathematical equations that describe the motion of water waves. Different equations represent different waves – waves coming onto a beach, waves in a puddle, or waves in your bathtub. Then when I've surfed the math, I like nothing better than to spend the rest of the day surfing the waves.

This field is very important. The better we can model water waves, the better we can predict the patterns of beach erosion and natural disasters such as the tsunami in South East Asia. And this research can be applied to all sorts of regions around the world.

Being a member of AAAS means I get to learn about areas of interest I might not otherwise encounter. It gives me valuable opportunities to exchange ideas with colleagues in other fields. And this helps me find new approaches to my own work.”

Dr. Katherine Socha is an assistant professor of mathematics at St. Mary’s College, Maryland. She’s also a member of AAAS.

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Gene Regulatory Networks and the Evolution of Animal Body Plans

Eric H. Davidson[1]* and Douglas H. Erwin[2]

Development of the animal body plan is controlled by large gene regulatory networks (GRNs), and hence evolution of body plans must depend upon change in the architecture of developmental GRNs. However, these networks are composed of diverse components that evolve at different rates and in different ways. Because of the hierarchical organization of developmental GRNs, some kinds of change affect terminal properties of the body plan such as occur in speciation, whereas others affect major aspects of body plan morphology. A notable feature of the paleontological record of animal evolution is the establishment by the Early Cambrian of virtually all phylum-level body plans. We identify a class of GRN component, the "kernels" of the network, which, because of their developmental role and their particular internal structure, are most impervious to change. Conservation of phyletic body plans may have been due to the retention since pre-Cambrian time of GRN kernels, which underlie development of major body parts.

Large gene regulatory networks (GRNs) that determine the course of animal development are now being decoded experimentally [e.g., (*1*–*8*)]. These networks consist largely of the functional linkages among regulatory genes that produce transcription factors and their target cis-regulatory modules in other regulatory genes, together with genes that express spatially important signaling components. They have a modular structure, consisting of assemblies of multigenic subcircuits of various forms. Each such subcircuit performs a distinct regulatory function in the process of development (*1*, *9*). GRN structure is inherently hierarchical, because each phase of development has beginnings, middle stages, and progressively more fine-scale terminal processes, so that network linkages operating earlier have more pleiotropic effects than those controlling terminal events. The earlier stages of formation of every body part involve specification of the domain of the developing organism that will become that part, followed by pattern formation, which determines its morphological structure. Only at the end of this process are deployed the differentiation gene batteries that encode the detailed functional properties of the body part (*10*).

The structure/function properties of developmental GRNs provide an approach to an old and general problem in animal evolution. What mechanisms account for the fact that has there has been so little change in phylum- and superphylum-level body plans since the Early Cambrian [e.g., the Chengjiang fauna (*11*–*13*)] (Fig. 1), though on the other hand, great changes have subsequently occurred within phyla and classes (e.g., the advent of tetrapod vertebrates, insects, dinosaurs, modern forms of echinoids, and cephalopods)? Furthermore, continuous modification characterizes the process of speciation. Classic evolutionary theory, based on selection of small incremental changes, has sought explanations by extrapolation from observed patterns of adaptation. Macroevolutionary theories have largely invoked multilevel selection, among species and among clades. But neither class of explanation provides an explanation of evolution in terms of mechanistic changes in the genetic regulatory program for development of the body plan, where it must lie.

Functional Properties of Diverse GRN Components

Change in the structure of the diverse kinds of subcircuits of which GRNs are constructed will have different consequences for the outcome of the developmental process, and therefore for evolution as well. Here we consider the following classes of GRN component: (i) evolutionarily inflexible subcircuits that perform essential upstream functions in building given body parts, which we term the "kernels" of the GRN; (ii) certain small subcircuits, the "plug-ins" of the GRN, that have been repeatedly coopted to diverse developmental purposes; (iii) switches that allow or disallow developmental subcircuits to function in a given context and so act as input/output (I/O) devices within the GRN; and (iv) differentiation gene batteries. These parts are illustrated in a real developmental GRN, the sea urchin endomesodermal GRN, in fig. S1.

Five properties can be used to define GRN kernels. First, these are network subcircuits that consist of regulatory genes (i.e., genes encoding transcription factors). Second, they execute the developmental patterning functions required to specify the spatial domain of an embryo in which a given body part will form. Third, kernels are dedicated to given developmental functions and are not used elsewhere in development of the organism (though individual genes of the kernel are likely used in many different contexts). Fourth, they have a particular form of structure in that the products of multiple regulatory genes of the kernel are required for function of each of the participating cis-regulatory modules of the kernel ("recursive wiring"). Hence, the fifth property of the kernel is that interference with expression of any one kernel gene will destroy kernel function altogether and is likely to produce the catastrophic phenotype of lack of the body part. The result is extraordinary conservation of kernel architecture.

Two examples of kernels illustrate many of these points (Fig. 2). Both display detailed conservation of complex subcircuit architectural structure across immense periods of evolutionary time, and both are surrounded by other network linkages that are not conserved. The first (Fig. 2A) includes a gene regulatory feedback loop required for endoderm specification in echinoderms that has existed at least since divergence at the end of the Cambrian half a billion years ago (*14*) and could, of course, be much older. The second (Fig. 2B) is a heart-field specification kernel (*15*) that must be even more ancient, as it is used in both *Drosophila* and vertebrate development. These subcircuits operate to specify the cellular populations where, respectively, the gut and the heart will form and to set up the regulatory states on which subsequent developmental processes will depend.

In the echinoderm endoderm network, five of the six genes in the kernel (all except *delta)* encode DNA-recognizing transcription factors; that is, they are regulatory genes, and this is true of all the genes in the conserved circuitry in the heart network. In both kernels, the linkages are highly recursive. For example, in the endoderm kernel, the cis-regulatory module of the *otx* gene receives input from three of the five genes; the *foxa* gene, from three of the five; and the *gatae*, *foxa*, and *bra* genes from two of the same five genes; similarly, in the vertebrate heart network, the *nkx2.5*, *tbx*, *mef2c*, and *gata4* genes all receive inputs from multiple other genes of the kernel, as do the *tin*, *doc*, *mid*, *pnr*, and *mef2c* genes of the *Drosophila* network. It is also the case that loss of expression of any of these genes in either kernel has a catastrophic effect on development of the respective body parts (*1*, *14*–*16*). There are a number of additional

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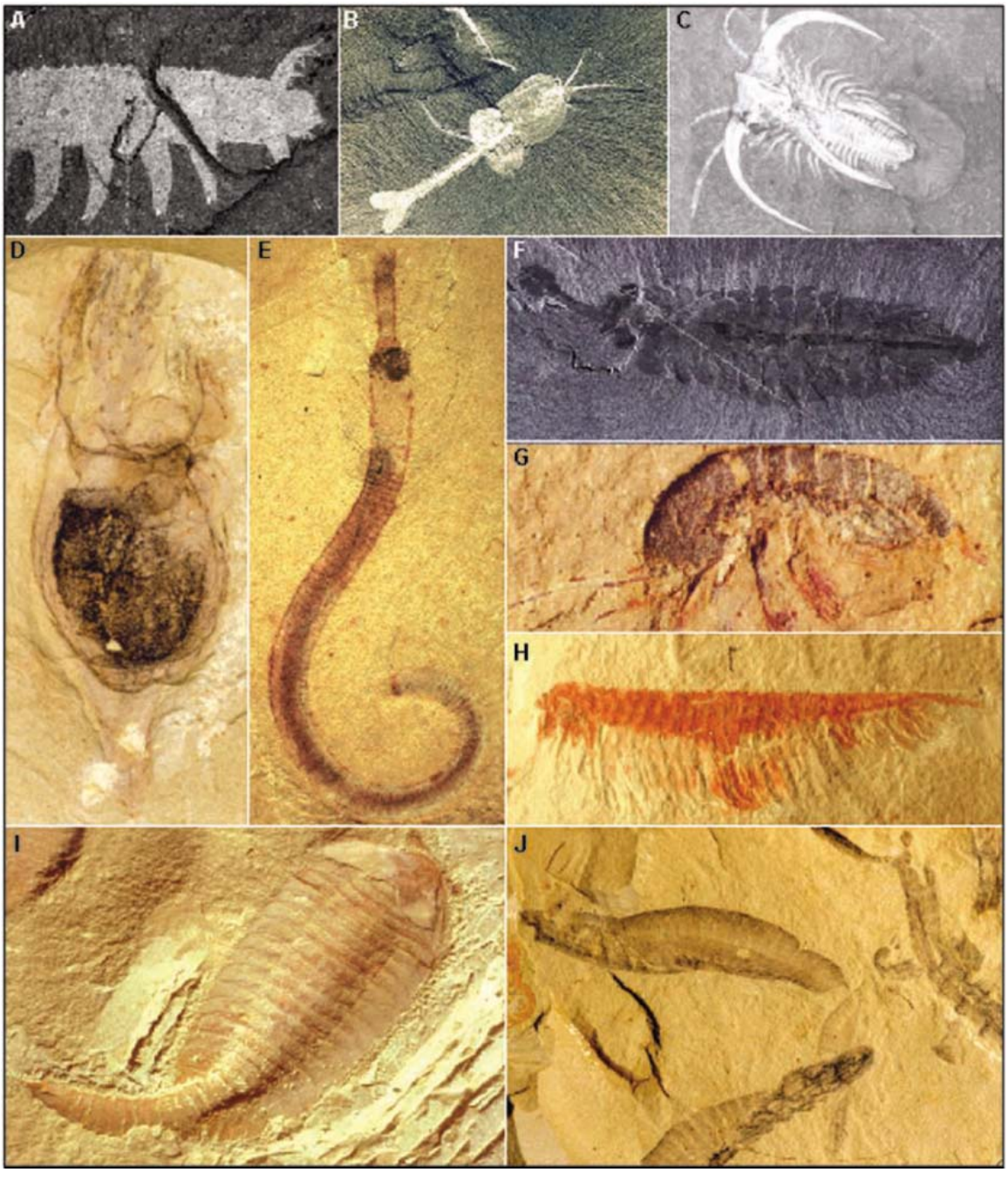


Fig. 1. Examples of Cambrian body plans from the Early Cambrian (~510 million years ago) Chengjiang Fauna of Yunnan Province, China (**D** to **I**) and the Middle Cambrian Burgess Shale Fauna of British Columbia, Canada (**A** to **C**, **J**). These fossils are the remains of animals all of which have body plans that can immediately be related to those of modern phyla, as indicated. For instance, the bilateral, anterior-posterior organization and position of the appendages in the arthropod examples resemble those of the modern counterparts; in addition, the chordate has a segmented dorsal muscular column and a notochord, as do modern chordates. (A) Onycophoran: *Aysheaia pedunculata*; (B) arthropod: *Waptia fieldensis*; (C) arthropod: *Marrella splendens*; (D) possible ascidian: *Phlogites*; (E) priapulid: *Maotianshania cylindrica*; (F) pan-arthropod: *Opabinia regalis*; (G) arthropod: *Leanchoilia illecebrosa*; (H) arthropod: *Jianfengia multisegmentalis*; (I) arthropod: *Fuxianjuia protensa*; (J) chordate: *Haikouella lanceolata*; [(A) to (C)] and (F) are from D. H. Erwin, Smithsonian Institution; (D), (E), and [(G) to (J)] are courtesy of J.-Y. Chen, Nanjing Institute of Geology and Palaeontology, China (*13*).

examples for which there is persuasive evidence for the existence of GRN kernels awaiting discovery of the direct genomic regulatory code. Prospective examples include kernels common to all members of a given phylum or superphylum required for the following: anterior to posterior (*17*–*19*) and midline to lateral (*20*, *21*) specification of the nervous system (in deuterostomes and possibly across Bilateria); eye-field specification [in arthropods (*22*, *23*)]; gut regionalization [in chordates (*24*, *25*)]; development of immune systems [across Bilateria (*26*, *27*)]; and regionalization of the hindbrain and specification of neural crest [in chordates (*28*, *29*)].

''Plug-ins'' also consist of structurally conserved GRN subcircuits, but as they are used for many diverse developmental functions within and among species, these network subcircuits are not dedicated to formation of given body parts. Instead, they are inserted in many different networks where they provide inputs into a great variety of regulatory apparatus. The best examples are signal transduction systems, of which a small set, each affecting a confined repertoire of transcription factors, are used repeatedly, often acting as dominant spatial repressors in the absence of ligand and as facilitators of spatially confined expression in its presence (*30*). In Bilateria, Wnt (*31*), transforming growth factor–β (TGF-β) (*32*), fibroblast growth factor (*33*), Hedgehog (*34*), Notch (*35*), and epidermal growth factor (*36*) signaling systems are used for myriad purposes during development. Their deployment is very flexible, and even in homologous processes in related animals these plug-ins may be used differently (*37*). Consider, for example, the several dozen different TGF-β genes in amniote vertebrates, expressed differentially in the (species-specific) terminal phases of development (*32*, *38*). It follows that their connections into the network are evolutionarily very labile.

Differentiation gene batteries are defined as groups of protein-coding genes under common regulatory control, the products of which exe-

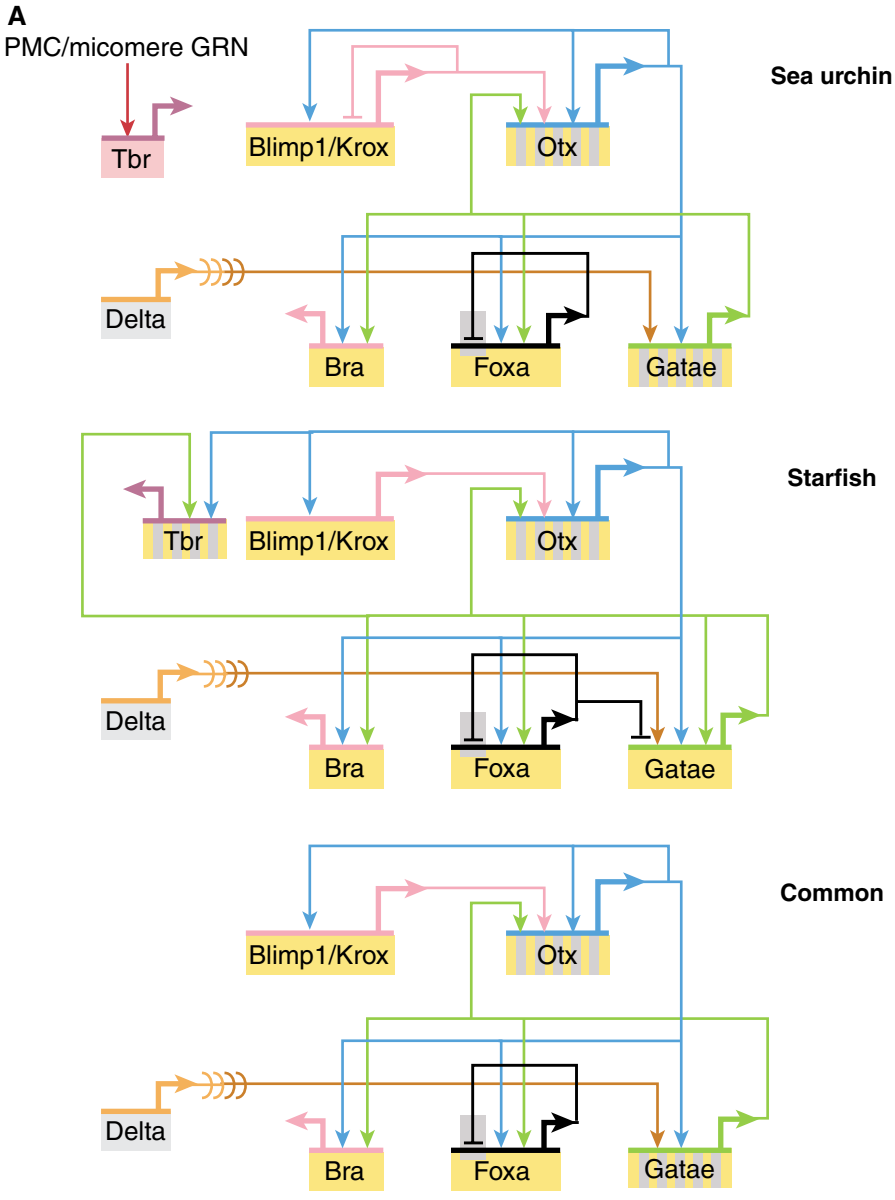
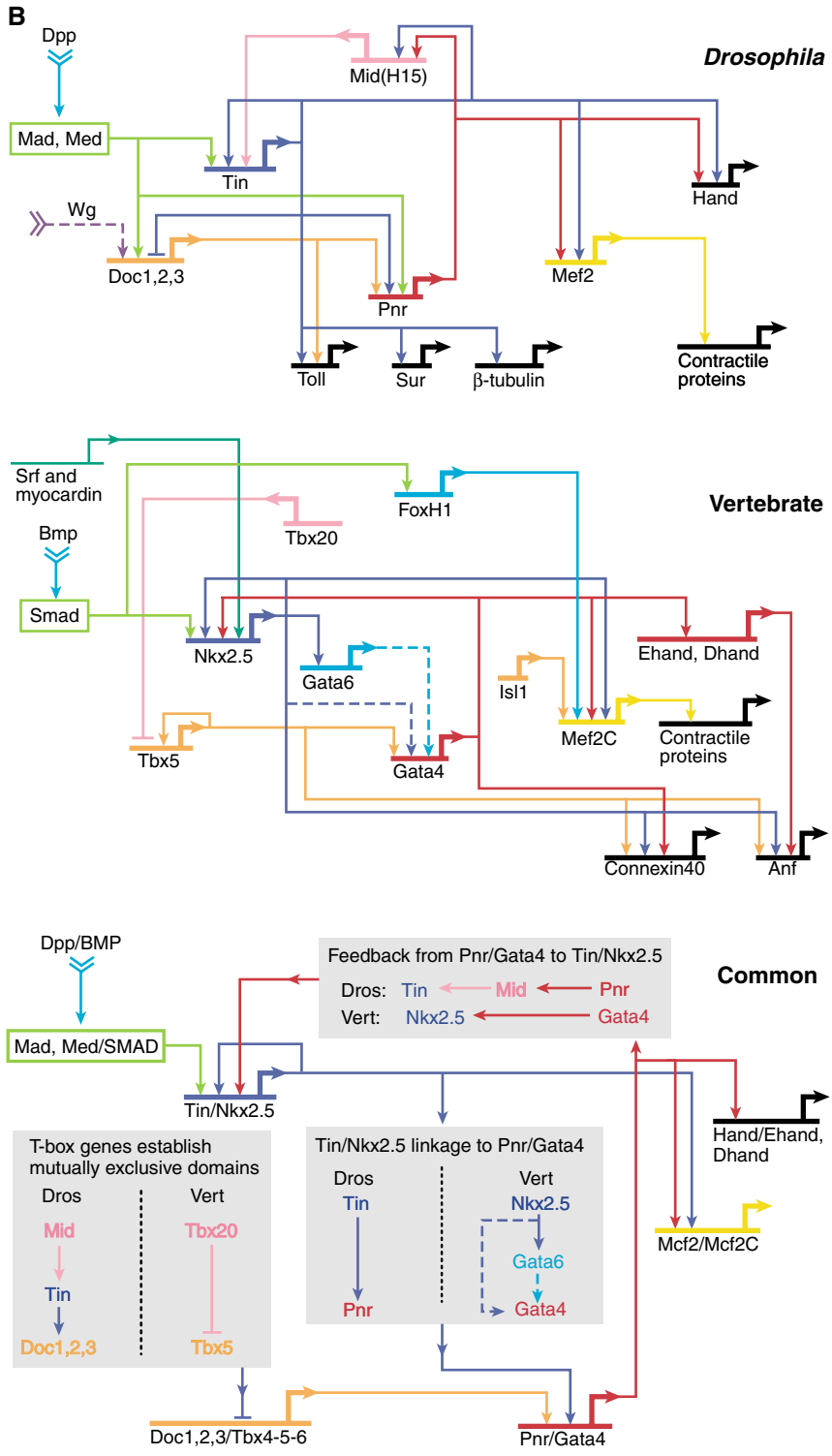


Fig. 2. Examples of putative GRN kernels. Networks were constructed and portrayed using BioTapestry software (*55*). (**A**) Endomesoderm specification kernel, common to sea urchin and starfish, the last common ancestor of which lived about half a billion years ago. The relevant area of the sea urchin network is shown at the top [(*1*, *9*, *16*); for currently updated version, details, and supporting data, see (*56*)]; the corresponding starfish network (*14*) is shown in the middle; and the network architecture, which has been exactly conserved since divergence—i.e., the kernel—is shown at the bottom. Horizontal lines denote cis-regulatory modules responsible for the pregastrular phase of expression considered, in endoderm (yellow), mesoderm (gray), or both endoderm and mesoderm (striped gray and yellow). The inputs into the cis-regulatory modules are denoted by vertical arrows and bars. The gray box surrounding the *foxa* input indicates that this repression occurs exclusively in mesoderm. (**B**) Possible heart specification kernels; assembled from many literature sources (*15*). Dashed lines show possible interactions. Some aspects of the GRN that may underlie heart specification in *Drosophila* are shown at the top; the approximately corresponding vertebrate relationships are shown in the middle; and shared linkages are shown at the bottom. Absence of a linkage simply means that this linkage is not known to exist, not that it is known not to exist. Many regulatory genes participate in vertebrate heart formation for which orthologous *Drosophila* functions have not been discovered, and the hearts themselves are of very different structure. However, as pointed out by many authors [see (*7*, *8*, *57*) for reviews of earlier references], a core set of regulatory genes are used in common and are now known to be linked in a similar way in a conserved subcircuit of the gene network architecture, as shown. The gray boxes represent in each case different ways that the same two nodes of the network are linked in *Drosophila* and vertebrates.

cute cell type–specific functions. Such functions contrast with those of kernels and plug-ins, the significance of which is entirely regulatory. Differentiation gene batteries build muscle cells and make skeletal biominerals, skin, synaptic transmission systems, etc. The structure of differentiation gene batteries has been discussed at the network level elsewhere (*10*); as an example, see the skeletogenic and pigment cell differentiation gene batteries in fig. S1. Differentiation gene batteries display inherent evolutionary lability and undergo continuous renovation. Numerous examples can be found in studies of speciation [e.g., (*39*)]. Internal changes occur in differentiation gene batteries in various ways: Any of the tens or hundreds of structural genes constituting their working components may alter functionally by incremental changes in their protein-coding sequences; new genes may be added to them if they acquire cis-regulatory modules targeting members of the given small set of transcriptional regulators to which that battery responds; or similarly, they may lose genes. But differentiation gene batteries reside at the periphery of developmental GRNs (*40*), because their outputs terminate the network. They are expressed in the final stages of given developmental processes. They do not regulate other genes, and they do not control the progressive formation of spatial patterns of gene expression that underlies the building of the body plan; in short, they do not make body parts. They receive rather than generate developmental instructions.

Cis-regulatory linkages that may be considered as I/O switches regulating other network subcircuits appear to be responsible for many kinds of evolutionary change in developmental process. For example, a common form of variation, which must be trivial at the regulatory level because it occurs even within genera and species, is in size of homologous body parts. We can easily imagine that this parameter depends only on the input linkage between a regulatory gene of the network controlling the patterning of the body part, and a cell cycle cassette; indeed, such linkages are explicitly known, for instance, in the gene network regulating pituitary development where the target is the cell cycle control genes (*41*). Here, the *pitx2* regulatory gene specifically activates the cell cycle control genes *cyclin D1*, *cyclin D2*, and c-*myc*. Many *hox* gene functions are also in this class: They act to permit or prohibit patterning subcircuits from acting in given regions of an animal. Examples include the direct repressive effects of the Ubx gene product on expression of wing-patterning genes in the *Drosophila* haltere (*42*–*44*); the role of group 10 and 11 hox genes in specification of vertebral morphol-



ogy in vertebrates (*45*); and, in beetles, the function of Ubx to allow the wing-pattern network to operate in the forewings, preventing expression of a different program expressed normally in the hindwing (*46*).

Predicted Evolutionary Consequences of Changes in GRN Architecture

Viewed in this way, it is apparent that the effects of changes in different component classes will be qualitatively distinct, causing disparate kinds of effect on body plan and on adaptive organismic functionality. Furthermore, there emerges a relation between the network-component class in which changes might occur and the taxonomic level of morphogenetic effects (Fig. 3).

The most frequent and least constrained kinds of change will occur in the peripheral regions of the GRN, i.e., within differentiation gene batteries themselves and the apparatus that controls their deployment. This is for the simple reason that there are no downstream consequences in cis-regulatory wiring elsewhere in the network if peripheral input linkages change, as will commonly result from change in more internal locations. Such peripheral, small changes are just what is observed in the countless processes of speciation. They account for many adaptive properties of the organism, for instance, different properties of the integument, the repertoire of digestive enzymes, the positioning of peripheral sensory elements, etc.

At the other extreme (Fig. 3) are the kernels of the network. They operate the peculiarly crucial step of specifying the domain for each body part in the spatial coordinate system of the postgastrular embryo. We think that change in them is prohibited on pain of developmental catastrophe, both because of their internal recursive wiring and because of their roles high in the developmental network hierarchy. We predict that when sufficient comparative network data are available, there will be found conserved network kernels similar in complexity and character to those of Fig. 2, which program the initial stages of development of every phylum-specific body part and perhaps of superphylum and pan-bilaterian body parts as well. It would follow that these kernels must have been assembled during the initial diversification of the Bilateria and have retained their internal character since. Critically, these kernels would have formed through the same processes of evolution as affect the other components, but once formed and operating to specify particular body parts, they would have become refractory to subsequent change. Molecular phylogeny places this evolutionary stage in the late Neoproterozoic when Bilateria begin to appear in the fossil record (*47*–*51*), between the end of the Marinoan glaciation at about 630 million years ago and the beginning of the Cambrian. Therefore the mechanistic explanation for the surprising fact that essentially no major new phylum-level body parts have evolved since the Cambrian may lie in the internal structural and functional properties of GRN kernels: Once they were assembled, they could not be disassembled or basically rewired, only built on to.

Between the periphery of developmental GRNs and their kernels lies the bulk of the network architecture. Here we see skeins of special cross-regulatory circuitry, plug-ins, and I/O connections; and here is where have occurred the changes in network architecture that account for the evolutionary novelties attested in the fossil record of animals.

Reinterpreting the Evolutionary Record

We propose that architectural changes in animal body plans have been produced over the past 600 million years by changes in GRNs of at least three general classes, with extremely different developmental consequences and rates of occurrence. This challenges the generally time-

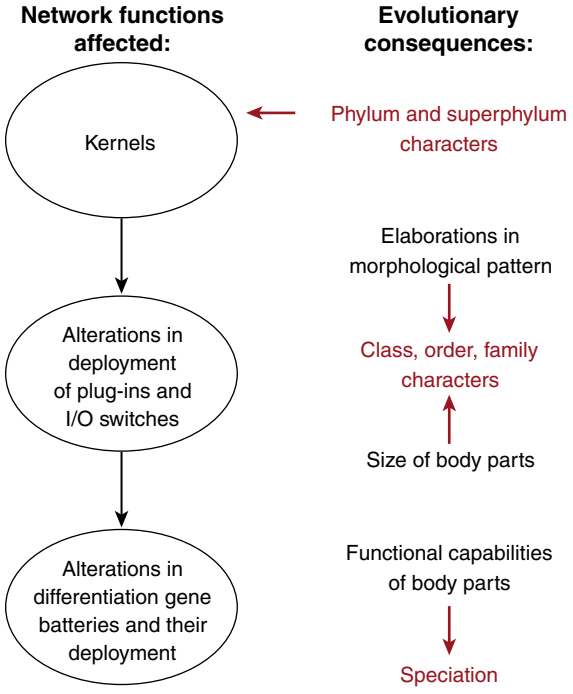


Fig. 3. Diverse kinds of change in GRNs and their diverse evolutionary consequences. The left column shows changes in network components; the right column shows evolutionary consequences expected, which differ in their taxonomic level (red).

homogeneous view of most evolutionary biologists. Current microevolutionary thinking assumes that observed types of genetic change (from single base substitutions to gene duplications) are sufficient to explain all evolutionary events, past and present. Such changes are considered as having occurred during evolution in a temporally homogeneous way. Microevolution does intersect with mechanisms of GRN change at the level of change within cis-regulatory modules. But attempting to explain an aspect of animal evolution that depends on one kind of network alteration by adducing evidence from an aspect that depends on another can be fundamentally misleading. Comparative molecular dissection of GRNs should allow identification of the evolutionary point of origin of each subcircuit and linkage in the network, and hence each morphological character of the body plan.

If the early assembly of kernels underlies the phyletic conservation of body parts since the Cambrian, then the position in GRNs of subsequent adaptational change is forced to lower levels in the network hierarchy. The result is what has been termed developmental or phylogenetic constraints (*52*–*54*). The different levels of change that have occurred in evolution are imperfectly reflected at different levels of Linnean classification, and we think that these inhomogeneous events have been caused by architectural alterations in different locations in the underlying GRNs. Following the early assembly of kernels, the varying effects of plug-in redeployment, changes in I/O linkages, and piecemeal alterations in differentiation gene batteries provide a basis for mechanistic analysis of subphyletic animal evolution. To the extent that kernel formation underlies critical morphological innovations, some kernels must indirectly be responsible for major events in Neoproterozoic niche construction. Motility, predation, digestion, and other canonical features of the Bilateria followed from the evolutionary appearance of the genetic programs for the respective body parts. These innovations became an engine of change that irreversibly altered the Earth's environment and, thus, the probability of success of subsequent evolutionary changes. We believe that experimental examination of the conserved kernels of extant developmental GRNs will illuminate the widely discussed but poorly understood problem of the origination of animal body plans in the late Neoproterozoic and Cambrian and their remarkable subsequent stability.

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page 806

INTRODUCTION

The Invisible Bouquet

OF THE THOUSANDS OF DIFFERENT METABOLITES THAT PLANTS CAN PRODUCE, many form a cloud around the plant. These volatile compounds reflect the metabolic complexity of plants and also serve a diversity of functions. Volatile compounds signal opportunity to insects, pathogens, and pollinators alike. In a classic case of "the enemy of my enemy is my friend," plants being nibbled on by insect herbivores can produce volatile signals that call in other insects to prey on the herbivores. For plants that flower at night, volatiles may be a better signal than floral color or shape to draw in the best insect pollinators. Volatile signals are also read by neighboring plants and reinterpreted as instructions to adjust their own defenses. Through olfaction and, secondarily, through the combination of taste and olfaction, which we interpret as flavor, volatiles signal to mammals that what's nearby may be food or foul. Some volatile compounds have biochemical functions, such as the antimicrobial activity of the spice clove. The prevalence of clove and similar spices in traditional food recipes has much to do with the value of these spices for preserving food in pre-refrigeration human history. That these spices also deliver a unique flavor through their volatility serves as well as an overt signal of the (hopefully) better quality of the food so prepared.

In this special issue, we explore various notes of this aromatic story. Lund and Bohlmann (p. 804) discuss how genome, environment, and cultivation practices shape the suite of volatiles that eventually give each bottle of wine its unique flavor. Kaiser (p. 806) illustrates how effectively certain plants and fungi can mimic each other, poaching on their insect partnerships by using volatile signals. Pichersky *et al.* (p. 808) describe the biochemistry and the evolutionary forces that combine to produce the complex suite of volatiles. Baldwin *et al.* (p. 812) explain how plants eavesdrop on their neighbors to adjust their own reactions to ecosystem changes. And finally, Goff and Klee (p. 815) put forth a hypothesis about how volatiles fine-tune or misdirect our human responses to food.

In related online resources, a Science of Aging Knowledge Environment (SAGE KE) Perspective by Rawson explores the nature of age-related olfactory loss and how it might be prevented. Information at *Science*'s Signal Transduction Knowledge Environment (STKE) highlights plant signaling by the volatile plant hormone jasmonate (see the Connections Maps by the Farmer lab), and a Teaching Resource by Laskowski shows an animated model of the regulation of gene expression by auxin. In an STKE Perspective, Vogt focuses attention on the evolution of olfactory receptors and the molecular mechanisms for perceiving odorants.

–PAMELA J. HINES

Plant Volatiles

CONTENTS

See also related SAGE KE, STKE, and ScienceCareers material on p. 735 or at www.sciencemag.org/sciext/plantvolatiles/

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PERSPECTIVE

The Molecular Basis for Wine Grape Quality—A Volatile Subject

Steven T. Lund[1]* and Joerg Bohlmann[2]

Volatile organic compounds are important flavor components of finished wines. In addition to winemaking practices, which shape wine quality, cultivation of the grape berries in the vineyard each season affects the production of volatile organic compounds as well as other chemical components that ultimately contribute to our perception of flavor in finished wines. By studying how berry flavor components are determined by the interplay of vine genotypes, the environment, and cultivation practices at the molecular level, scientists will develop advanced tools and knowledge that will aid viticulturalists in consistently producing balanced, flavorful berries for wine production.

Many of us like to relax with a nice glass of wine, but have you ever considered the complex chemistry at play on your nose and palette when you first raise the glass? Whether you find the bouquet and entry from a newly opened bottle to be a pleasant experience or the basis for a scowl and a wrinkle of the nose partly depends on the relative assortment of volatile compounds in the wine. The many volatile organic compounds (VOCs) and other chemical compounds contributing to flavor (taste and aroma) in wines are determined in part in the vineyard through a complex and poorly understood interplay between the natural environment, vineyard management practices, and vine genotypes, including the rootstocks (*1*, *2*). Thus, the consistent production of high-quality grapes for winemaking has traditionally been more art than science. This art is increasingly guided by science for many wine producers, and this trend will continue with a growing contribution from molecular-based technologies and knowledge. Here, we focus on viticulture and grape berry biochemistry, but we acknowledge that enology (the science of winemaking) and human olfactory reception also play critical roles in determining flavor and individual human perception of quality in wines.

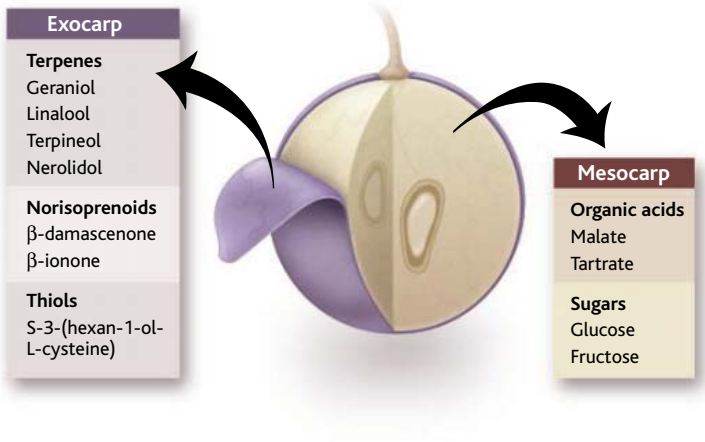

Fig. 1. Major chemical determinants of flavor and wine quality in grape berries are predominantly localized to mesocarp (flesh) or exocarp (skin) tissues. Only a small number of the dozens of known grape compounds important for flavor are represented here. Potentially volatile compounds such as terpenes, norisoprenoids, and thiol precursors are stored as sugar or amino acid conjugates in vacuoles of exocarp cells. The compounds are volatilized through physical crushing and subsequent cleavage by grape, yeast, and/or industrial enzymes (glycosidases and peptidases) during the winemaking process.

Metabolic changes throughout the biphasic growth of grape berries lay the groundwork for flavor. After flowering and fruit set in the first phase, there is an initial burst of growth in the pericarp (flesh plus skin) and seed. The accumulation and storage of organic acids, chiefly malate and tartrate, in mesocarp (flesh) cell vacuoles occurs during this time. The tartness imparted by these acids in the pericarp likely evolved as a safeguard against mammalian and avian foraging while the seeds developed. The first phase is followed by a lag period in which expansion slows while seed maturation is completed. Finally, the second phase of berry ripening is initiated and maturation of the pericarp begins—a process termed "veraison" by the French. Maturation is marked in red cultivars by accumulation of anthocyanins (red pigments) in the exocarp (skin), down-regulation of glycolysis coupled with glucose and fructose accumulation, metabolism of malate as the major carbon source for respiration, and biosynthesis of VOCs and other metabolites important for flavor (*3*). Thus, after seed maturation, the berry becomes more visually attractive and flavorful, promoting geographical seed distribution by foraging animals. For winemaking, harvest dates are chosen to optimize the balance between sweetness, acidity, flavorfulness, and phenolic ripeness. Harvest for winemaking usually occurs 12 to 14 weeks after fruit set.

The most important grapevine compounds contributing to flavor are organic acids, proanthocyanidins (tannins), terpenoids (monoterpenoids, sesquiterpenoids, and C13-norisoprenoids), and various precursors of aromatic aldehydes, esters, and thiols detectable in finished wines. Glucose, fructose, malic acid, and tartaric acid are stored primarily in the vacuoles of mesocarp cells, although some glucose and fructose can be detected in the exocarp. Proanthocyanidins and other polyphenolic compounds, terpenoids, esters, and other less abundant sensory compounds are primarily stored in exocarp cells (Fig. 1). Plant-derived volatile terpenoid compounds occurring in wines are mainly stored as nonvolatile, water-soluble glycoside derivatives (sugar conjugates) in exocarp cell vacuoles, although some terpenoids may also be present as free volatiles. Unlike other aromatic plants that sequester large amounts of lipophilic terpenoid VOCs in specialized anatomical structures (such as glandular trichomes on the surfaces of peppermint leaves, glandular structures in the peels of citrus fruits, or resin

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ducts in pine bark), grape berries seem to lack such anatomical structures for physical sequestration of lipophilic VOCs. Instead, conjugation as water-soluble glycosides provides an alternate means of biochemical trapping and storage of the VOCs. Other important volatiles for wine flavor may be stored as amino acid conjugates in grape cells, such as cysteinylated precursors of aromatic thiols (*4*). Volatilization of compounds stored as conjugates in grape cells is essential for our perception of these as flavor. Glycosidases and peptidases, enzymes that cleave the sugar and amino acid conjugates, thus play a vital role in the timing and production of natural fruit flavors. For winemaking, physical crushing and processing through fermentation can introduce grape and yeast enzymes, respectively, to conjugated substrates. Winemakers may also add exogenous enzyme mixtures to the fermentation to further stimulate volatilization of compounds to improve sensory characteristics of VOCs in the finished wines (*5*, *6*).

The relative assortment of compounds in the berries of each grape variety define what is known as "varietal character" to wine enthusiasts. An excellent example is the vegetative character conferred to Sauvignon Blanc grapes and wines by methoxypyrazine compounds, chiefly 2-methoxy-3-isobutylpyrazine (*7*). We are fine-tuned to perceive methoxypyrazines and can detect them at parts per trillion (femptomolar) levels, possibly as a deterrent to feeding on unripe, acidic fruits. Unlike most compounds important for flavor, methoxypyrazines accumulate during green stages of berry development and are gradually metabolized during maturation, the extent to which is dependent upon sun exposure and other microclimate factors. Subtle herbaceousness is generally held as a desirable character in Sauvignon Blanc wines, whereas it can be considered a defect in most red varietals such as Merlot. In contrast, dozens of different terpenoid compounds contribute nuances of floral or fruity characters to wines, depending on the varietal. Muscat varieties such as Gewürztraminer, for example, are rich in monoterpene compounds, chiefly geraniol and citronellol, which contribute a distinctive floral character to the wines (*8*). Linalool is another monoterpene compound that not only imparts a floral character to berries but has also been implicated in flower aroma as well as a signaling response to insect feeding, suggesting multiple biological roles for this VOC in plants. One of the most important norisoprenoid compounds for red wine quality is β-damascenone, which imparts a honeylike, fruity character at femptomolar levels (*9*).

There may be dozens to hundreds of chemical compounds in grape berries that, similar to the methoxypyrazines and norisoprenoids, exist in exceedingly small quantities but have yet to be discovered and characterized. Advances in extraction protocols and analytical techniques with the use of increasingly sensitive detection equipment technologies such as Fourier transform ion cyclotron resonance (FT-ICR), mass spectrometry, and nuclear magnetic resonance are pushing the envelope of plant metabolomics and will undoubtedly aid in the discovery of new compounds in grape berries. Relatively few of the dozens of *Vitis* (grapevine) species have been domesticated by humans for wine production. Metabolomics research in grapes should not be limited to commercially important wine grape cultivars but could be extended to further characterize the rich diversity inherent amongst *Vitis* species (*10*). In considering such analyses in grapes, however, human sensory analyses should continue to be coupled with the discovery of new compounds and analogs of known ones in order to characterize if and how they impact berry flavor (*11*). The same considerations should be made for metabolomic analyses of yeast compounds or grape compounds modified by yeasts and detected in finished wines.

An important current research challenge is determining how environmental cues affect the regulation of the genes and enzymes of various metabolic pathways leading to the diverse bouquet of VOCs and other important compounds for wine flavor. Previous experiments have shown how varied environmental conditions affect berry ripening and quality at harvest. The limitation is that such research has been focused on cause and effect—i.e., the "what"—but the mechanisms underlying these processes—i.e., the "how"—remain largely unknown and unexplored. A better understanding of how temperature, light, and water and nutrient availability to the berry qualitatively and quantitatively affect allozyme (i.e., enzyme variant) production and activity will help to develop molecular diagnostic tools that will assist viticulturalists in fine-tuning pruning, cluster thinning, irrigation, and fertilization practices from season to season in each vineyard. To achieve this, complex networks of signaling and metabolic pathways must be characterized at the gene, protein, and metabolite levels in varied, controlled environments in order to begin to clarify how accumulation of sensory compounds is regulated at the molecular level in the grapevine. As a first step, gene cloning and functional characterization of enzymes important for the formation of VOCs in grapes have recently been reported (*12*–*14*). As with other systems of plant VOC formation, the numerous terpenoid synthase (TPS) (*12*, *13*) and carotenoid cleavage dioxygenase (CCD) (*14*) enzymes likely orchestrate much of the complex metabolic profiles of terpenoid or isoprenoid VOCs in *Vitis* species. Curiously, TPSs contribute not only to berry VOC formation but also to the diurnal emissions of VOCs from grapevine flowers. A functional, biochemical genomics approach of TPS and CCD discovery should support metabolite profiling and molecular mapping of related grape quality traits.

Recent and ongoing molecular investigations hold promise in revealing the biological secrets underlying berry ripening and flavor that are currently inaccessible to viticulturalists and winemakers. We expect that increased mechanistic knowledge and the development of new molecular tools (e.g., DNA and protein chip–based technologies) will help guide viticulturalists in clonal selection (matching genotypes to specific mesoclimates), as well as vineyard management to more consistently produce high-quality grapes for wine production from season to season.

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PERSPECTIVE

Flowers and Fungi Use Scents to Mimic Each Other

Roman Kaiser

Some flowering plants mimic the scent and appearance of mushroom fruiting bodies. Fungi may also mimic flowers. In addition, infection of plants by certain fungi can direct the plant to develop nonfunctional floral-like structures that nonetheless primarily serve the reproductive advantage of the fungus. These various mimicries may serve to attract insects that in turn spread fungal spores or plant pollen, thus facilitating sexual reproduction of the cryptic organism.

The ultimate purpose of the scent of a flower, in conjunction with its shape and coloring, is to attract the respective pollinators to ensure the preservation of the species. Some of these scents are delightful, as for night-scented moth flowers. Others are putrid, as for flowers that mimic carrion or dung to attract certain fly species that deposit their eggs in the flowers, thereby acting as pollinators. The interchange serves only the flower, however, as the fly larvae usually cannot develop. Such so-called sapromyophilous flowers occur in primitive as well as in highly advanced angiosperms (*1*).

Fungus gnat flowers also attract flies to their flowers. They mimic the fruiting bodies of mushrooms by scent and morphological appearance. Fly species that normally deposit their eggs in mushrooms are attracted to these flowers and thus pollinate them. Flowers of the genera *Asarum* and *Aristolochia* (Aristolochiaceae), *Arisarum* and *Arisaema* (Araceae), and *Dracula* (Orchidaceae) use this strategy (*2*).

The extraordinarily shaped flowers of *Dracula chestertonii* (Fig. 1), native to the Colombian Andes, emit a mushroom-like scent assumed to attract females of a fungus fly species. The large lip imitates the lamellated cap of fungi so well that the fungus fly deposits its eggs in the fake mushroom cap and incidentally effects pollination of this orchid (*2*). The floral fragrance of *D. chestertonii*, which corresponds to what people recognize as champignon scent, includes the typical mushroom constituents oct-1-en-3-ol (**1**), oct-1-en-3-one (**2**), octan-3-ol (**3**), and octan-3-one (**4**), making up more than 70% of the volatiles (*3*, *4*) (Fig. 2). The flowers of the orchid *D. vampira*, endemic to Mount Pichincha in Ecuador, also emit a mushroom scent of comparable olfactory quality to *D. chimaera*.

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Another fascinating example of a fungus gnat flower is that of *Aristolochia arborea*, a small tree from the rainforest of Central America (*2*). The flowers appear at the base of the stem in short branched inflorescences. The perianth forms a flytrap typical of Aristolochiaceae. These flowers present a perfect imitation of a small toadstool in the center of the brown perianth tube. The "toadstool" resembles a member of the genus *Marasmius* (*5*), fungi that grow in tropical rainforests. The flowers of *A. arborea* emit a rather faint meaty, earthy, and mushroom-related scent. Because of the very low scent emission, we have so far been able to identify only the main constituents of the floral scent of *A. arborea*: the terpenoids α-pinene, camphene, β-pinene, sabinene, limonene, β-cedrene, caryophyllene, germacrene D, bicyclogermacrene, germacrene A, and germacra-1(10),5-dien-4-ol.

Fungi, just as flowering plants, may use mimicry to steal resources to ensure survival. An impressive example is that of the witches' brooms on *Berberis vulgaris*, which are induced by the systemically infecting rust fungus *Puccinia arrhenatheri* (*6*). *B. vulgaris* is the alternate host of *P. arrhenatheri* and at least five other *Puccinia* spp., the wheat rust (*P. graminis*) included, which all infect grass species as their primary host. Thus, barberry shrubs are eradicated in areas with intensive wheat cultivation. During the spermatic stage of *P. arrhenatheri*, the infected leaves of *B. vulgaris* reveal a yellowish color, reminiscent of flower petals, and emit a strong floral-fruity and herbaceous scent, which is mainly due to indole (**5**), methyl nicotate (**6**), carvacryl methyl ether (**7**), (*Z*)-7-decen-5-olide (**8**, jasmine lactone), (*Z*)-7-decen-4-olide (**9**, γ-jasmolactone) (*6*), and its precursor (*E*,*Z*)-5,7-decadien-4-olide (**10**) (Fig. 3). Of these, only traces of indole were found in the uninfected leaves, which give off no more than 2% of the quantity of volatiles given off by either infected leaves or flowers. The bright yellow color and the strong scent of infected leaves mimic flowers and attract a wide diversity of insects, which facilitate the sexual reproduction of the pathogen by transporting spermatia (*6*).

Fungal infection inhibits the barberry shrub from blooming. The flowers of uninfected *B. vulgaris* open later in the season, when the fungus has terminated the production of spermatia. By contrast to the flowery-fruity fragrance of witches' brooms, *B. vulgaris* flowers emit a peculiar spermy odor even though it is based on pleasant odorants such as linalool and isomers of lilac aldehyde and lilac alcohol (*6*). The spermy odor aspect frequently encountered in flower scents is due to minor amounts of 1-pyrroline (**11**) and traces of 2-acetyl-1-pyrroline (**12**) (Fig. 3).

Another fascinating example of floral mimicry by a plant pathogenic fungus is the systemic infection of the Canada thistle *Cirsium arvense* by the fungus *P. punctiformis* (*7*). During its spermatial stage, the fungus on the infected thistle shoots emits a strong floral scent that is mainly based on phenylacetaldehyde, 2-phenylethyl alcohol, and indole.

Floral mimicry can address both scent and shape so much that it convinces even the human eye. Rust fungi in the *P. monoica* complex infect 11 species of Brassiaceae, mainly North American *Arabis* species, modifying host leaf morphology to produce fungal "pseudoflowers" that aid the sexual reproduction of the fungus (*8*). "Pollinator" attraction is accomplished through visual floral mimicry, the presence of nectar reward, and floral fragrances. The pseudoflower scent does not appear to represent a simple modification of host floral or vegetative emission, nor does it mimic the scent of co-

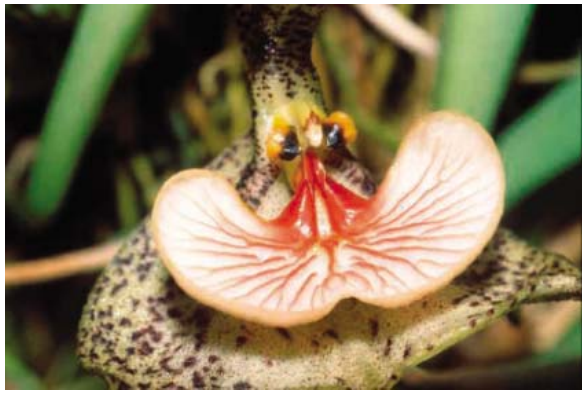

Fig. 1. The unusually large flower lip of *Dracula chesteronii* (Rchb.f.) Luer, an orchid native to the Colombian Andes, mimics by scent and appearance the fruiting body of a mushroom to attract fungus gnats (width of lip, 2.5 cm).

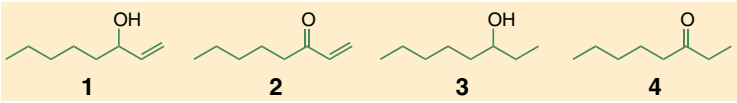

Fig. 2. Typical mushroom constituents dominating the flower scent of *Dracula chestertonii* (Rchb.f.) Luer.

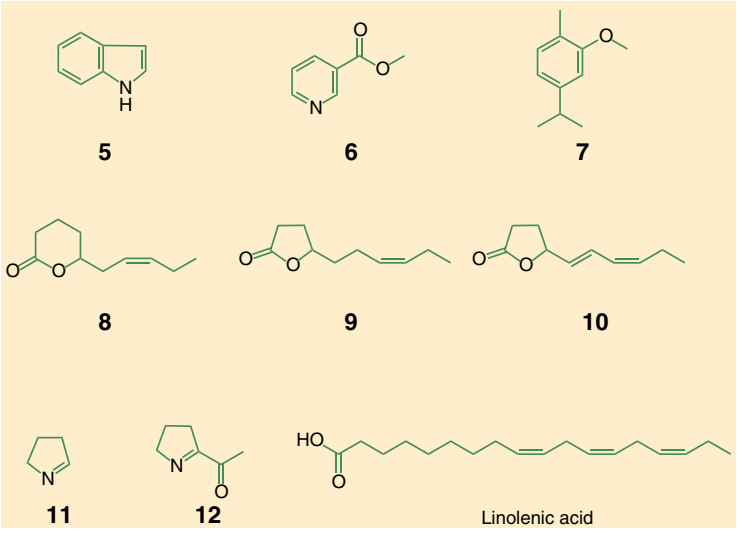

Fig. 3. Typical scent constituents emitted from pathogen specimens of *Berberis vulgaris* L., *Euphorbia cyparissias* L., and *Euphorbia verrucosa* L.

Fig. 4. Flowering plants of *Euphorbia cyparissias* L. together with specimens infected by a rust fungus of the species complex *Uromyces pisi* and having developed so-called pseudoflowers (width of rosette, 1.5 cm).

blooming flowers. Instead, the unique fragrances, beyond their function as pollinator attractants, may reduce gamete loss by reinforcing constancy among foraging insects (*9*, *10*). The distinct scent of these fungal pseudoflowers is composed primarily of aromatic compounds such as 2-phenylethyl alcohol and its esters as well as phenylacetaldehyde (*10*), compounds also found in thistle when infected by *P. punctiformis* (*7*).

Impressive pseudoflowers, yellow leaves that grow in a dense terminal rosette and resemble true flowers in color and shape (*11*) (Fig. 4), are also formed by the systemic infection of *Euphorbia cyparissias* by rust fungi of the species complex *Uromyces pisi*, the latter being common pathogens of *E. cyparissias* in Europe (*12*). Like true flowers, they present scent and sweet-smelling nectar on the surface, which contains fungal gametes that are transferred by nectar-feeding insects from one fungal mating type to the other (*11*). The true flowers of *E. cyparissias* are characterized by a green, aromatic-floral, honey-like odor mainly consisting of mono- and sesquiterpenes, lipid metabolites such as (*Z*)-hexen-3-yl acetate, and aromatic compounds including phenylacetic acid. By contrast, the scent given off by rust-induced pseudoflowers is distinct and variable, most likely depending on the taxonomic affiliation of the fungal parasite and perhaps also on the maturity of the pseudoflower.

We found that pathogen specimens from Oberstammheim (northeast Switzerland) had a strong spermy scent containing at least 30% 1-pyrroline (**11**), but those near Birchwil (northeast Switzerland) and in other habitats had the fruity-floral scent of peach, mainly caused by jasmine lactone (**8**) and indole (**5**) (Fig. 3). To make it even more complicated, other *Euphorbia* species may be infected by *Uromyces* species and develop similarly looking pseudoflowers. Near Merishausen (northeast Switzerland), *E. cyparissias* co-occurs with *E. verrucosa*, but only the latter species was infected and developed pseudoflowers induced by *U. euphorbiae-Craccae* (*12*), which emitted again a distinct floral-fruity scent, this time dominated by a new scent compound of molecular weight 168. We isolated this compound and elucidated its structure by nuclear magnetic resonance as (*E*,*Z*)-5,7-decadien-4-olide (**10**), the biological precursor of the widespread (*Z*)-7-decen-4-olide (**9**). Structural considerations suggest that **10** as well as **9** and the δ-analog **8**, including derivatives, are biogenetically derived from linolenic acid. In fact, the lactones **10** and **9** are formed from linolenic acid by biodegradation (*13*). Linolenic acid is metabolized, probably by *U. euphorbiae-Craccae*, in pseudoflowers of *E. verrucosa* mainly to γ-lactones (e.g., **10**, **9**) and in those of *U. pisi s. lat* on *E. cyparissias* exclusively to δ-lactones (e.g., **8**), whereas *P. arrhenatheri* on *B. vulgaris* (*6*) activates both pathways.

The olfactory mimicry that these fungi and flowers use to their advantage coincidentally produces scents that elicit strong human reactions, whether of disgust or delight. We choose the latter as the base for new perfumes.

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REVIEW

Biosynthesis of Plant Volatiles: Nature's Diversity and Ingenuity

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Plant volatiles (PVs) are lipophilic molecules with high vapor pressure that serve various ecological roles. The synthesis of PVs involves the removal of hydrophilic moieties and oxidation/hydroxylation, reduction, methylation, and acylation reactions. Some PV biosynthetic enzymes produce multiple products from a single substrate or act on multiple substrates. Genes for PV biosynthesis evolve by duplication of genes that direct other aspects of plant metabolism; these duplicated genes then diverge from each other over time. Changes in the preferred substrate or resultant product of PV enzymes may occur through minimal changes of critical residues. Convergent evolution is often responsible for the ability of distally related species to synthesize the same volatile.

Plant volatiles (PV) are typically lipophilic liquids with high vapor pressures. Nonconjugated PVs can cross membranes freely and evaporate into the atmosphere when there are no barriers to diffusion. The number of identified volatile chemicals synthesized by various plants exceeds 1000 and is likely to grow as more plants are examined with new methods for detecting and analyzing quantities of volatiles that are often minute (*1*–*3*).

PVs serve multiple functions in both floral and vegetative organs, and these roles are not always related to their volatility (*1*). Most PVs are restricted to specific lineages and are involved in species-specific ecological interactions, leading to their designation as specialized, or secondary, metabolites (*4*). To humans, pollinator-attracting floral scents have been a source of olfactory pleasure since antiquity, and we also use a large number of aromatic plants as flavorings, preservatives, and herbal remedies (*5*). Flower and herb aromas may contain many individual chemicals, sometimes with very little overlap in the PV profiles of even closely related species (*6*). It is unlikely that the observed differences are due entirely to differential gene expression. That most PVs are restricted to a few lineages, both ancient and derived, argues for frequent changes in enzymatic profiles through evolution. Considerable diversity and fast rates of change have also been observed in nonvolatile specialized metabolites in plants, raising similar questions regarding the underlying evolutionary mechanisms (*7*).

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Improvements in analytical techniques and molecular and biochemical methods have made PVs one of the best-studied groups of plant secondary metabolites. Here we describe insights into molecular mechanisms involved in the biosynthesis of PVs and their implications for our understanding of plant metabolic diversity.

PV Biosynthetic Pathways Branch Off from Primary Metabolism

The largest class of PVs is derived from isoprenoid pathways. In plants, both the cytosolic mevalonate and the plastidic methylerythritol phosphate pathways generate the five-carbon compound isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). A plastidic prenyltransferase synthesizes geranyl pyrophosphate (GPP) from the condensation of one IPP molecule and one DMAPP molecule. A second type of plastidic prenyltransferase condenses DMAPP with three IPP molecules to produce geranylgeranyl pyrophosphate (GGPP). In the cytosol, the condensation of one DMAPP molecule with two IPP molecules results in farnesyl pyrophosphate (FPP). These pyrophosphate-containing compounds serve as precursors of many primary metabolites (*8*).

Plants also have enzymes called terpene synthases (TPSs), which catalyze the formation of diverse hemi-, mono-, sesqui-, and diterpene PVs from DMAPP, GPP, FPP, and GGPP, respectively (*9*). Many distinct TPSs that synthesize monoterpenes and sesquiterpenes (the bulk of terpenoid PVs) have been characterized from various plants (*1*, *2*, *10*). Each species contains a number of these mechanistically and structurally related TPSs. For example, the *Arabidopsis* genome contains 32 genes that appear to encode functional TPSs, including six proven monoterpene synthases and at least two proven sesquiterpene synthases (*11*).

The first step in the reaction catalyzed by TPSs is removal of the pyrophosphate group, leading to a carbocation (Fig. 1A). This highly unstable intermediate can then undergo a number of multistep transformations. The active site of each TPS channels and directs the intermediates through regio- and stereochemical rearrangements to produce often one or two major products and a few (or even scores of) minor "derailment" products (*9*, *11*, *12*). For example, four residues lining the active-site cavities of two highly related maize TPSs, designated TPS4 and TPS5, control the proportions of sesquiterpenes produced in these enzymes (Fig. 1) (*12*). Changing only these four residues in TPS5 to those found in TPS4 produced a volatile profile and catalytic efficiency identical to that of native TPS4, even though differences elsewhere in the proteins remained (*12*).

The second largest class of PVs consists of compounds containing an aromatic ring. Although not all reactions leading to the synthesis of the basic skeletons have been determined, most are derived from intermediates in the pathway that leads from shikimate to phenylalanine and then to an array of primary (such as lignin) and secondary nonvolatile compounds (*2*). Eugenol (clove essence) is a reduced version of coniferyl alcohol, a lignin precursor (*13*). Phenylacetaldehyde, a compound present in tomato fruit (*14*), is derived from phenylalanine by decarboxylation and oxidative removal of the amino group (*15*). Shortening of the three-carbon side chain of phenylalanine-derived hydroxycinnamates to one carbon also leads to aromatic building blocks such as benzoic acid and benzaldehyde (*16*). Still other PVs are produced by type III polyketide synthases (PKSs) that use cinnamoyl- and malonyl–coenzyme As (CoAs) as starting material (*17*).

A third group of PVs is derived by oxidative cleavage and decarboxylation of various fatty acids, resulting in the production of shorter-chain volatiles with aldehyde and ketone moieties (*18*, *19*) that often serve as precursors for the biosynthesis of other PVs (*20*). Similarly, some volatile terpenes are not derived directly from isoprenoid pyrophosphates but instead from the cleavage of carotenoids by carotenoid cleavage dioxygenases (CCDs) (*21*). Other PVs, particularly those containing nitrogen or sulfur, are synthesized by cleavage reactions of modified amino acids or their precursors (*5*). For example, the volatile indole is made in maize by the cleavage of indole-3-glycerol phosphate, an intermediate in tryptophan biosynthesis (*22*).

Some Modifying Enzymes Can Use Multiple Substrates

A common property of enzymes for specialized metabolites, including PVs, is their proclivity to act on multiple substrates. This promiscuity probably serves as an important cornerstone in the rapid functional divergence of such enzymes. For enzymes with broad substrate specificity, such as some carboxyl methyltransferases and acyltransferases, the availability of a substrate determines the type and amount of formed products. For example, petunia flowers contain a carboxyl-

methyltransferase that favors salicylic acid over benzoic acid but produces methylbenzoate in planta due to a lack of salicylic acid (*23*). In contrast, *Stephanotis floribunda* flowers contain an enzyme with similar properties, but both methylsalicylate and methylbenzoate are produced because both acid substrates are available (*24*).

Enzymes for the Biosynthesis of PVs Often Belong to Large Families

Specific chemical modifications can convert nonvolatile compounds to volatile ones, enhance the volatility of already volatile chemicals, and alter their olfactory properties. This kind of biosynthetic tailoring is responsible for the combinatorial diversity of non–isoprenoid-derived PVs and also contributes to increasing the number of terpenoid PVs (*2*).

Sequence analyses of whole genomes and of gene transcripts derived from plant tissues rich in PVs, combined with characterization of the generation of PVs by enzymes encoded by some of these genes, show that plants possess several families of modifying enzymes that catalyze similar reactions but use distinct substrates. For example, the enzymes that methylate the carboxyl group of salicylic and benzoic acids mentioned above are homologous to jasmonic acid carboxylmethyltransferase, producing the PV methyljasmonate found in both floral and vegetative tissues (*25*). Another family of modifying enzymes consists of acyltransferases, many of which catalyze the transfer of an acetyl group from acetyl-CoA to alcohols such as geraniol (geranyl acetate is a distinct "note" in the scent of roses) and 3-*cis*-hexen-1-ol (3-*cis*-hexenyl acetate is emitted from the crushed leaves of many species) (*20*, *26*). Enzymes in this family also catalyze the transfer of other acyl groups; for example, from benzoyl-CoA to benzyl alcohol to produce benzylbenzoate in flowers of *Clarkia breweri*, a California annual (*20*). Members of another large group of enzymes, the oxidoreductases, have been found to catalyze the oxidation of the monoterpene carveol to carvone (caraway spice) and the reduction of medium-chain fatty acid aldehydes to alcohols (*27*, *28*).

PV Modification Enzymes Often Evolve from Non-PV Enzymes

The genome of *Arabidopsis* contains 272 genes belonging to the cytochrome P450 oxidase family. A few such P450s participate in PV biosynthesis, such as the oxidative cleavage of fatty acids to produce volatile aldehydes, whereas others contribute to primary metabolism or nonvolatile secondary metabolism (*18*, *29*). Given the extreme diversity of P450s in *Arabidopsis*, we can expect to find similarly large P450 groups in other plants. Some of these enzymes probably function in PV biosynthesis. Indeed, a P450 responsible for the hydroxylation of the monoterpene limonene in the menthol pathway in mint has been described (*30*). The families of oxidoreductases, methyltransferases, and acetyltransferases may be similarly diverse (*2*).

In these protein families, little correlation is observed between the level of sequence similarity among enzymes and the structural similarity of their substrates. An illustrative example involves a group of *O*-methyltransferases that catalyze the formation of scent compounds in rose, basil, and *C. breweri* (Fig. 2). In rose flowers, 1,3,5-trimethoxybenzene is produced from phloroglucinol (1,3,5-trihydroxybenzene) through three successive methylations. An enzyme, designated phloroglucinol *O*-methyltransferase (POMT) monomethylates phloroglucinol but lacks iterative activity with subsequent methylated intermediates (*31*). The second and third reactions are catalyzed by two highly similar proteins (96% amino acid sequence identity), OOMT1 (orcinol *O*-methyltransferase 1) and OOMT2 (*32*). OOMT1 slightly prefers the first intermediate (1-methoxy-3,5-dihydroxybenzene) and OOMT2 prefers the second intermediate (1,3-dimethoxy-5-hydroxybenzene). The high degree of sequence identity between OOMT1 and OOMT2 indicates recent gene duplication and

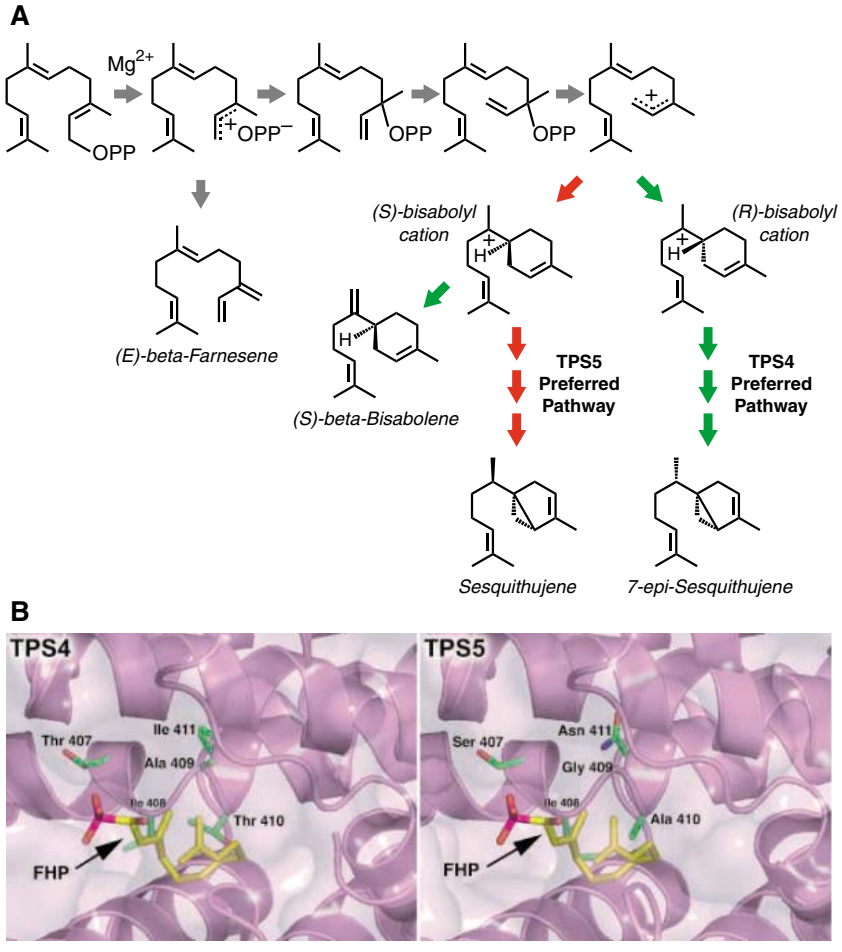

Fig. 1. Maize TPS4 and TPS5 catalyze the formation of the same complement of sesquiterpene products, albeit in distinctly different proportions. **(A)** Abbreviated TPS4 and TPS5 mechanisms accounting for the major products of each enzyme [29% (*S*)-β-bisabolene, 9% (*E*)-β-farnesene, 24% 7-epi-sequithujene, and 6% sesquithujene for TPS4; and 27% (*S*)-β-bisabolene, 13% (*E*)-β-farnesene, 2% 7-epi-sesquithujene, and 28% sesquithujene for TPS5]. For clarity, only the major products are shown or those products whose levels are considerably different for TPS4 and TPS5. OPP signifies a pyrophosphate. Gray arrows depict pathways equally shared among TPS4 and TPS5. Red and green arrows depict TPS5- and TPS4-preferred pathways, respectively [adapted from (*12*)]. **(B)** Active site models of TPS4 and TPS5 based on the crystal structure of tobacco 5-epi-aristolochene synthase (*10*), shown with the FPP nonhydrolyzable analog farnesyl hydroxyphosphonate (FHP). The four key residues responsible for functional divergence—Thr^{407}, Ala^{409}, Thr^{410}, and Ile^{411} in TPS4, and Ser^{407}, Gly^{409}, Ala^{410}, and Asn^{411} in TPS5—are shown as color-coded sticks, with the underlying secondary structure of the three-dimensional models colored lavender.

incipient gene evolution. However, neither of these two enzymes methylates phloroglucinol. POMT is highly divergent from OOMT1 and OOMT2 (30% identity), having higher sequence similarity (54% identity) to IEMT (isoeugenol/eugenol methyltransferase), the enzyme that methylates eugenol (and isoeugenol) in *C. breweri*. Moreover, POMT and IEMT have higher sequence identity (>60%) with COMT (caffeic acid *O*-methyltransferase), a methyltransferase involved in lignin biosynthesis (*31*, *33*). On the other hand, OOMT1 and OOMT2 are more similar (50% identical) to EOMT (eugenol *O*-methyltransferase), the basil enzyme that methylates eugenol, as well as to enzymes involved in isoflavone biosynthesis (*34*).

In vitro mutagenesis experiments with these and similar enzymes show that changes in a few critical residues can create PV biosynthetic enzymes with altered substrate specificity (*33*, *34*). For example, a single active-site substitution in basil chavicol *O*-methyltransferase (CVOMT) (Fig. 2) creates an enzyme whose substrate specificity is identical to that of basil EOMT (*34*). The presence of gene families enhances the chances for duplications to occur and also increases the chances of repeated evolution, a special case of convergent evolution in which enzymes with similar functions evolve independently in different species from homologous but not necessarily orthologous genes (such as eugenol methyltransferases in basil and *C. breweri*) (*4*). On the other hand, because a new volatile may confer higher fitness than an existing volatile, gene duplication is not an absolute prerequisite for divergence, and orthologous genes in different species may encode enzymes for different PVs (*35*).

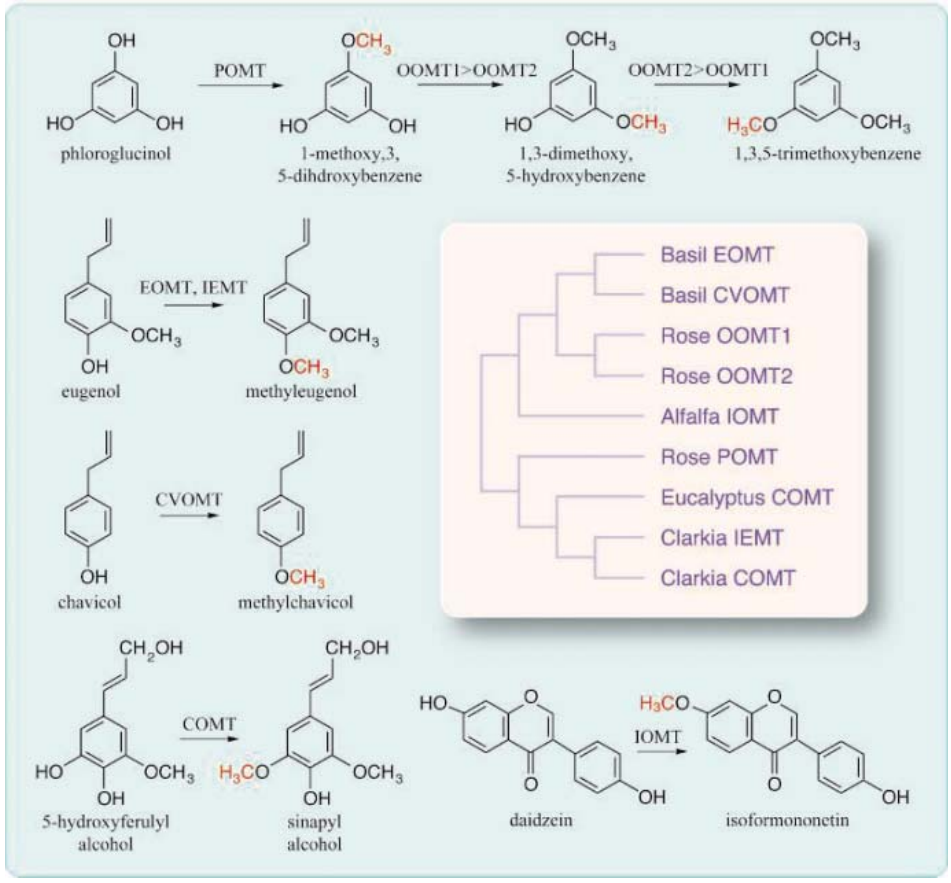

Fig. 2. Structural relatedness of some methyltransferases and their substrates. POMT, OOMT1, and OOMT2 are involved in synthesizing the rose floral volatile 1,3,5-trimethoxybenzene. Basil EOMT and *C. breweri* IEMT methylate eugenol to produce methyleugenol. Basil CVOMT methylates chavicol to produce methylchavicol. Eugenol, chavicol, methylchavicol, and methyleugenol are all volatiles with distinct aromas. COMT is an enzyme in the lignin biosynthetic pathway, and IOMT methylates the nonvolatile isoflavone daidzein to the nonvolatile isoformonentin. All these enzymes use *S*-adenosyl-L-methionine as the methyl donor [adapted from (*31*–*34*)]. The branches in the schematic phylogenetic tree are not drawn to scale.

Spatial and Temporal Modulation of PV Biosynthesis

In flowers, the biosynthesis of PVs usually occurs in epidermal cells (Fig. 3A), allowing an easy escape of PVs into the atmosphere (*36*). In vegetative organs, PVs may be synthesized in surface glandular trichomes (Fig. 3B) and then secreted from the cells and stored in a sac created by the extension of the cuticle (*13*, *37*). Some PVs are made in internal structures such as individual specialized cells (*38*) or ducts (*39*) from which they can be released upon disruption (for example, by herbivory). Free PVs in cells probably accumulate in membranes, but in some cases volatiles are glycosylated and apparently stored in vacuoles (*40*). The postsynthesis subcellular localization of PVs is, unfortunately, a much neglected area.

The rates of biosynthesis of leaf PVs are typically highest when leaves are young and not fully expanded and need the most protection, which PVs provide directly because of their toxicity or indirectly through the summoning of herbivores' predators (*1*, *2*, *41*). High rates are also observed when flowers are ready for pollination, and they decrease drastically after fertilization (*2*, *23*). Biosynthetic rates are correlated with levels of transcripts of genes encoding the final biosynthetic enzymes, or the concentration of the substrates of these enzymes, or both (*2*, *42*).

PV synthesis or emission may be influenced by environmental factors such as light, temper-

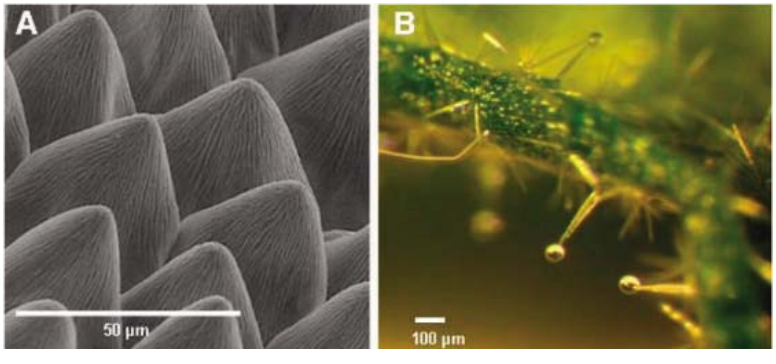

Fig. 3. Localization of storage and synthesis of PVs. (**A**) Conical cells on the surface of snapdragon petals synthesize and emit terpenes and benzenoids. (**B**) Glands of *Cistus creticus*, a shrub native to Crete, are rich in volatile and nonvolatile terpenes.

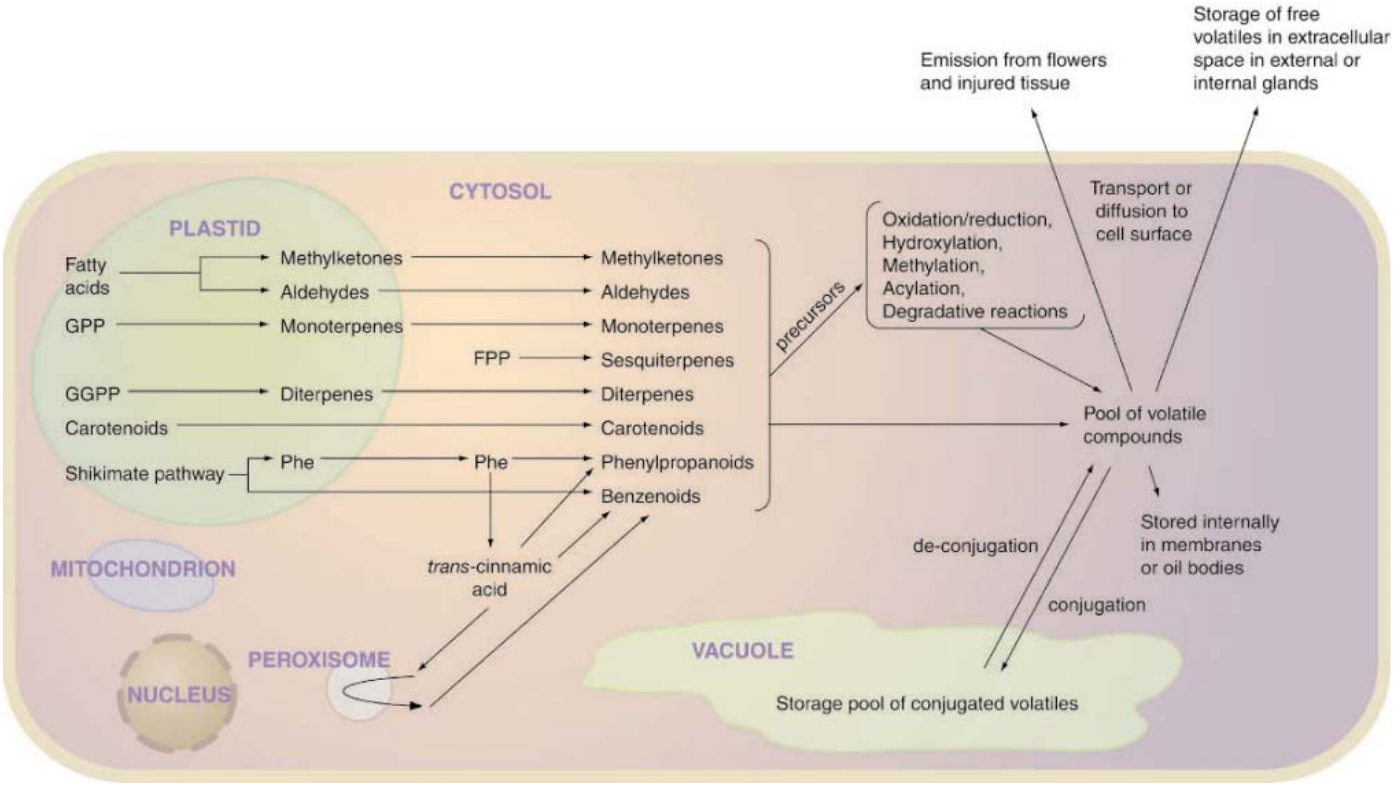

Fig. 4. Summary of the cellular processes involved in the synthesis of PVs. Most modification reactions occur in the cytosol, but some may take place in other subcellular compartments, including plastids, mitochondria, peroxisomes, and the endoplasmic reticulum.

ature, and moisture (*41*, *43*) and often follow a rhythmic pattern, which may be regulated by a circadian clock or light (*2*, *44*).

Conclusion

Each plant species is capable of synthesizing a unique set of volatiles. In the past decade, a large number of pathways and enzymes for the synthesis of PVs have been discovered (Fig. 4). We have learned that PVs are often derived from primary metabolism through the emergence of one or a few enzymes with new substrate specificities, mostly through duplication and divergence of enzymes used elsewhere in primary or secondary metabolism. Despite this progress, the enzymes responsible for the majority of already known PVs have not been characterized, and no doubt the number of PVs and their biosynthetic enzymes yet to be identified exceeds the known ones many times. Much remains to be elucidated regarding the internal and external factors influencing PV biosynthesis. Finally, the transport, storage, and emission of these compounds are neglected areas of study that must be addressed to complete our understanding of how plants use these specialized metabolites in diverse ecosystems.

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REVIEW

Volatile Signaling in Plant-Plant Interactions: "Talking Trees" in the Genomics Era

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Plants may "eavesdrop" on volatile organic compounds (VOCs) released by herbivore-attacked neighbors to activate defenses before being attacked themselves. Transcriptome and signal cascade analyses of VOC-exposed plants suggest that plants eavesdrop to prime direct and indirect defenses and to hone competitive abilities. Advances in research on VOC biosynthesis and perception have facilitated the production of plants that are genetically "deaf" to particular VOCs or "mute" in elements of their volatile vocabulary. Such plants, together with advances in VOC analytical instrumentation, will allow researchers to determine whether fluency enhances the fitness of plants in natural communities.

Plants excel at gas exchange: They can literally build forests from CO_2 taken from the air at about 120 Pg C $year^{-1}$, half of which is respired back to the atmosphere. Up to 36% of the assimilated carbon is released as complex bouquets of VOCs (*1*). Although some of these VOCs may be mere waste, others mediate various pollination and defense mutualisms with animals. These VOC-mediated interactions of plants with organisms of higher trophic levels suggest that they communicate similarly with each other (*2*). Two decades ago, researchers serendipitously discovered changes in herbivore resistance and secondary metabolites in plants ("receivers") growing adjacently to herbivore-attacked plants ("emitters"). Because in some experiments results were best explained by the aerial transfer of information (*3*), the phenomenon was popularly dubbed "talking trees." This phrase seems unfortunate, because selection most likely favors plants that "eavesdrop" on VOCs released from neighbors and respond by tailoring their phenotypes to enhance their own fitness.

What Are Plants Talking About?

An obvious conversation topic concerns impending attack from mobile herbivores, and most VOC-elicited responses have been interpreted accordingly. Measures of herbivore performance have been broadened to include the elicitation of various direct plant defenses (e.g., phenolics, alkaloids, terpenes, and defense proteins). Indirect defenses have also attracted attention, including food rewards that increase predation pressure on herbivores (*4*) and VOCs that help predators or parasitoids locate feeding herbivores (*5*, *6*). Moreover, the signal cascades that elicit direct and indirect defenses have been scrutinized (*7*, *8*) as have transcriptional responses (*9*–*12*) (Fig. 1).

VOC exposure alone, without actual herbivore attack, may directly increase the production of defenses. Alternatively, VOC exposure may allow nearby plants to ready their defenses for immediate use once the herbivores move from the neighboring plant to attack the "listening" receiver. Exposure to volatiles from damaged sagebrush primes the elicitation of defensive proteinase inhibitors (PIs) in wild tobacco, and exposed plants subsequently receive less damage (*13*–*15*) (Fig. 2). Corn seedlings previously exposed to either individual components or to the entire blend of VOCs released from herbivore-attacked seedlings responded to simulated herbivory with increased VOC production and higher jasmonate (JA) accumulations compared with the responses of unexposed plants (*8*). Whether these enhanced VOC emissions protect corn seedlings remains to be determined. The priming of defense cascades may benefit plants that would incur fitness costs by activating defense responses (*16*), particularly in the absence of herbivore attack (*17*). If VOC exposure directly elicited defense responses, receiver plants would incur similar fitness costs without being damaged. Hence, plants that avoided investing fitness-limiting resources in the production of costly defenses before an herbivore arrives, but were able to prime defense metabolism to launch defense responses when attacked, could realize a fitness benefit over plants that "ignored" the information coded in the VOCs emanating from their damaged neighbors.

The use of microarrays that monitor a large fraction of the plant's transcriptome can free analysis from observer bias about plants' conversation topics and identify selective pressures other than impending attack from mobile herbivores, which volatile signaling could be used to anticipate. Herbivores frequently transmit pathogens, and the elicited responses may concern attack by impending pathogens more than attack by herbivores (*18*). The relentless competition with other plants for resources that cannot be readily hoarded (such as light and nutrients) is likely the most important selective force for plants. Plants are able to anticipate impending competition through far red (FR) light signals and changes in the photon flux of blue light transmitted through their neighbors' canopies. These light signals are perceived by photoreceptors (e.g., phytochrome B) and elicit a complex of traits known as the shade-avoidance syndrome (SAS) (*19*). Experiments with tobacco plants transformed with a mutant ethylene receptor (*etr1-1*), which inhibits ethylene perception, have demonstrated that ethylene-insensitive tobacco could not respond rapidly to FR signals and consequently was outcompeted by wild-type plants (*20*). At concentrations apparently possible in dense plant canopies, ethylene by itself elicits the SAS (*21*). Similarly, exposure to unidentified VOCs from barley cultivars changes the allocation of biomass between roots and shoots without influencing biomass production of receiver barley genotypes (*22*), a re-allocation that may influence competitive ability. Thus, responses to the most important environmental factors in a plant's life may be anticipated by signals from neighboring plants. Almost anything can be a signal as long as it can be perceived and provides reliable information.

What Does It Take to Be a Signal?

Four steps characterize the transfer of VOC signals between plants: the release of the signal by the emitter plant and its transport, absorption, and perception by the receiver plant (Fig. 1). All are influenced by the signal's properties and its biological context. Most research on signal release has focused on the activation of biosynthetic enzymes and their substrate supply. The biochemical control mechanisms for the major VOC constituents are rapidly being clarified (Fig. 1). However, the release of foliar VOCs is also controlled by their physicochemical properties (*23*): Volatility is deter-

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mined by partitioning the compound between the "liquid" phase of the leaf and the atmosphere, whereas molecular size and stomatal aperture constrain diffusive transport from the leaf into the air surrounding the leaf, its headspace. Once released into the headspace of the emitter, the potential signal has to be transported to receivers. Direction and dynamics of this transport are dictated by temperature, convective transport, and wind for above-ground signaling or water for below-ground signaling. Small highly volatile compounds (e.g., ethylene, methanol, isoprene, acrolein, methacrolein, and some monoterpenes) diffuse rapidly into the headspace and are diluted in the atmosphere (Fig. 2). For such compounds, signaling function is likely limited to the foliage of the emitter (as a systemic within-plant signal) and of neighbors with intertwined canopies. Heavier compounds with less volatility, such as terpene alcohols, methyl jasmonate (MeJA), aromatic compounds including methyl salicylate (MeSA), and green-leaf volatiles (GLVs), are more likely to function as signals over longer distances, because their comparatively slower dispersal allows development of plumes of higher concentrations (*24*) that may be carried farther as intact parcels by turbulent flow (Fig. 2). During transport, some VOC species are oxidized or otherwise processed in the atmosphere (*1*), possibly causing dilution but also activation. The concentration gradients, which ultimately regulate the receiver's exposure, remain largely uncharacterized. An example of a characterized concentration gradient comes from a study of corn seedlings that release the volatile sesquiterpene (*E*)-β-caryophyllene into the soil from their roots, a below-ground plume used by entomopathogenic nematodes to locate root-attacking beetle larvae (*25*).

Signal volatility and diffusion rates, as well as the stomatal conductance of receiver plants, define the last steps in the signal transfer process: adsorption at the plant surface and uptake into the leaf via stomatal openings or cuticle diffusion. The low concentration gradient between atmosphere and leaf during the adsorption step amplifies the effects of the signal's physicochemical properties. Transport into the receiver leaf is influenced by stomatal conductance. The limited air volume of a sealed chamber increases VOC concentrations and also reduces CO_2 once the chambers are illuminated because of photosynthetic carbon fixation. Under such conditions, plants increase the number of open stomata, enhancing exposure of mesophyll cells to the VOCs. Therefore, sealed chambers are likely to influence the responsiveness of receiver plants, and studies that use them are more likely to report ecologically insignificant results.

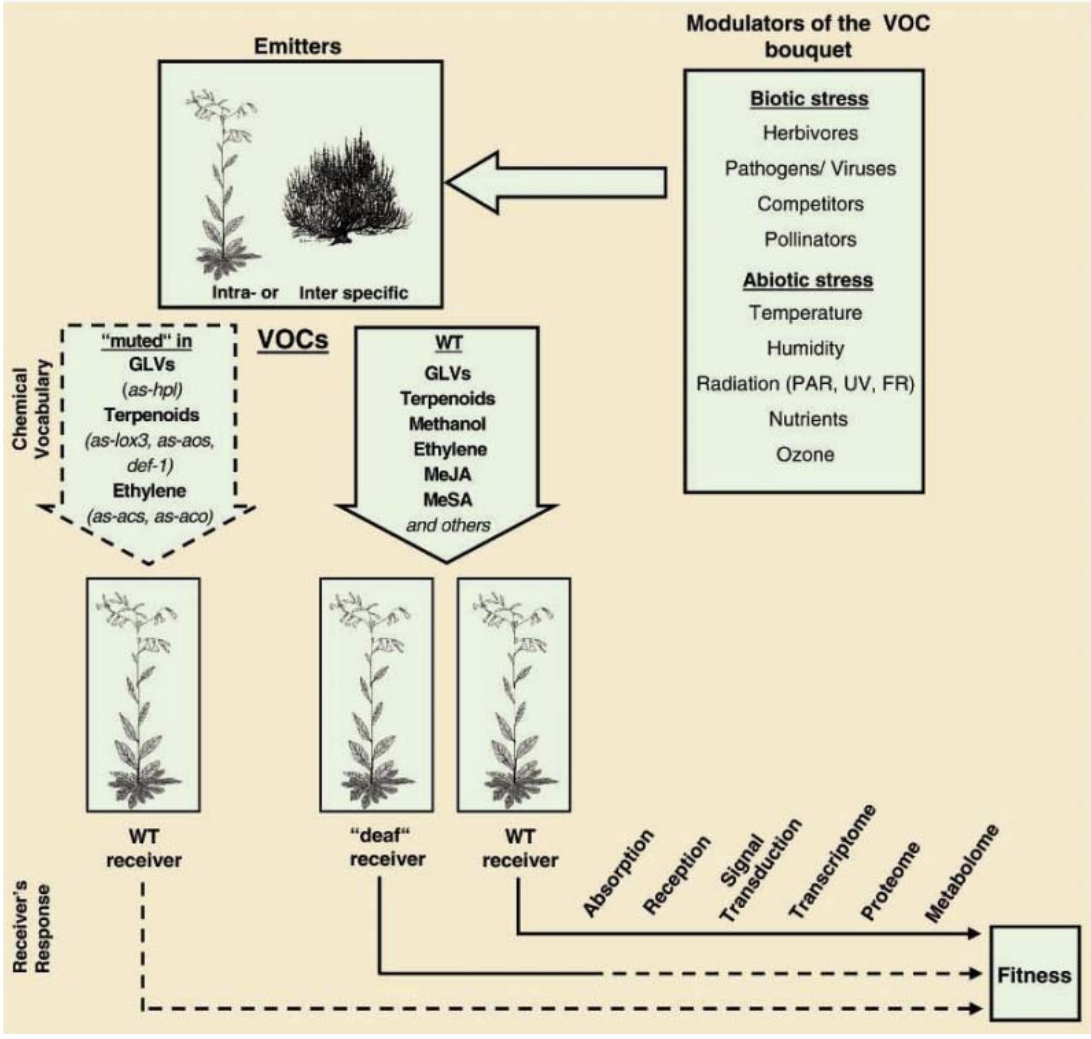

Fig. 1. Scheme of plant-plant interaction mediated by VOCs emphasizing the use of genetically manipulated plants to investigate the mechanisms underlying this process. Plants (e.g., wild tobacco) can be exposed to VOCs released from either conspecifics or from emitters of different species (e.g., sagebrush). The VOC bouquet of stressed plants consists of GLVs, terpenoids, MeJA, MeSA, methanol, ethylene, and other substances (*32*). Various biotic and abiotic stress factors modulate the chemical vocabulary emitted in quantity, quality, and timing. If the signal is recognized by the receiver plant, it may respond with changes in its signal transduction, transcriptome, proteome, and metabolome, which may or may not result in functionally significant changes in its fitness (→). Comparing responses to wild-type (WT) emitter plants with responses to mute emitters (⇢) whose VOC bouquet is deficient in one or more VOCs allows researchers to identify compounds mediating the interaction between emitters and receivers. In addition to insertional mutants [e.g., *def-1* (*33*)], various transgenic lines are generated by the expression of endogenous genes in antisense (as) orientations to silence enzymes necessary for eliciting or synthesizing VOCs, such as hydroperoxide lyase [HPL (*34*, *35*)], lipoxygenase [LOX3 (*36*)], allene oxide synthase [AOS (*35*)], 1-aminocyclopropane-1-carboxylic acid synthase [ACS (*37*)], or 1-aminocyclopropane-1-carboxylic acid oxidase [ACO (*38*)]. These lines represent possible mute emitters. Deaf receiver plants, such as the *etr1-1* line (*39*) impaired in functional VOC receptors for individual substances, could be used to verify each individual VOC's bioactivity.

Once a VOC enters the leaf, a response will only occur if the compound is "active," a poorly understood condition. Several proposed between-plant signals have hormone or hormone-like functions, including MeSA (*26*), GLVs (*8*, *9*, *27*), ethylene (*28*), and MeJA (*29*). However, proof that any of these are released and transported to receiver plants in quantities sufficient to elicit responses under natural conditions is either lacking or belies a signal function (*10*, *13*, *30*). Although most studies of bioactivity have examined whether the presence of a VOC elicits a response, removing certain components from a volatile bouquet can also elicit a response. The removal of GLVs from the wound-induced volatile blend by silencing hydroperoxide lyase strongly influenced the regulation of gene expression in neighboring conspecific tobacco plants (*10*). In other words, plants may respond to the "sounds of silence."

A class of electrophilic α,β-unsaturated carbonyl compounds represents potent regulators of gene expression (*11*). Although exposure to these highly volatile compounds increased the production of endogenous phytohormones, their activity was partially independent of the JA, SA, and ethylene signal cascades. A redox-based signal process, generated by the depletion of cellular reductants resulting from the electrophile reactivity of these compounds, suggests a mechanism for their activity that resembles the activation of the regulatory protein for pathogen defense, NPR1 (*31*). Similar processes may provide the basis of a general chemical "sense," which may have predated the evolution of receptors for particular volatiles.

Ecological Realism: "Deaf" and "Mute" Plants to the Rescue

Constitutive and herbivore-induced VOC emissions are influenced by a variety of abiotic factors [nutrient availability, temperature, wind, ultraviolet (UV) radiation and photosynthetically active radiation (PAR), and ozone exposure]. To lessen this variability, most studies of plant-plant signaling have been performed in the laboratory under experimental conditions (sealed or low air-flow chambers) that maximized the probability of detecting responses in receiver plants by increasing exposure [reviewed in (*10*)]. Although this work has shown that plants respond to being fumigated, its ecological relevance will remain unclear until the responses are verified in open-grown plants.

One solution to the problems of ecological realism in between-plant signaling studies is to use mutants or transgenic plants whose ability to either release or perceive particular components of the wild-type volatile blend is deficient. The use of "mute" emitters (*10*) allows complex herbivore-induced VOC blends to be dissected (Fig. 1). Complementation studies, in which synthetic constituents supplement the volatile blend to determine whether the receivers' response is subsequently restored, confirm function. The biosynthetic pathways contributing constituents to the herbivore-induced volatile bouquet and their regulatory cascades represent possible genetic targets. Mutants whose

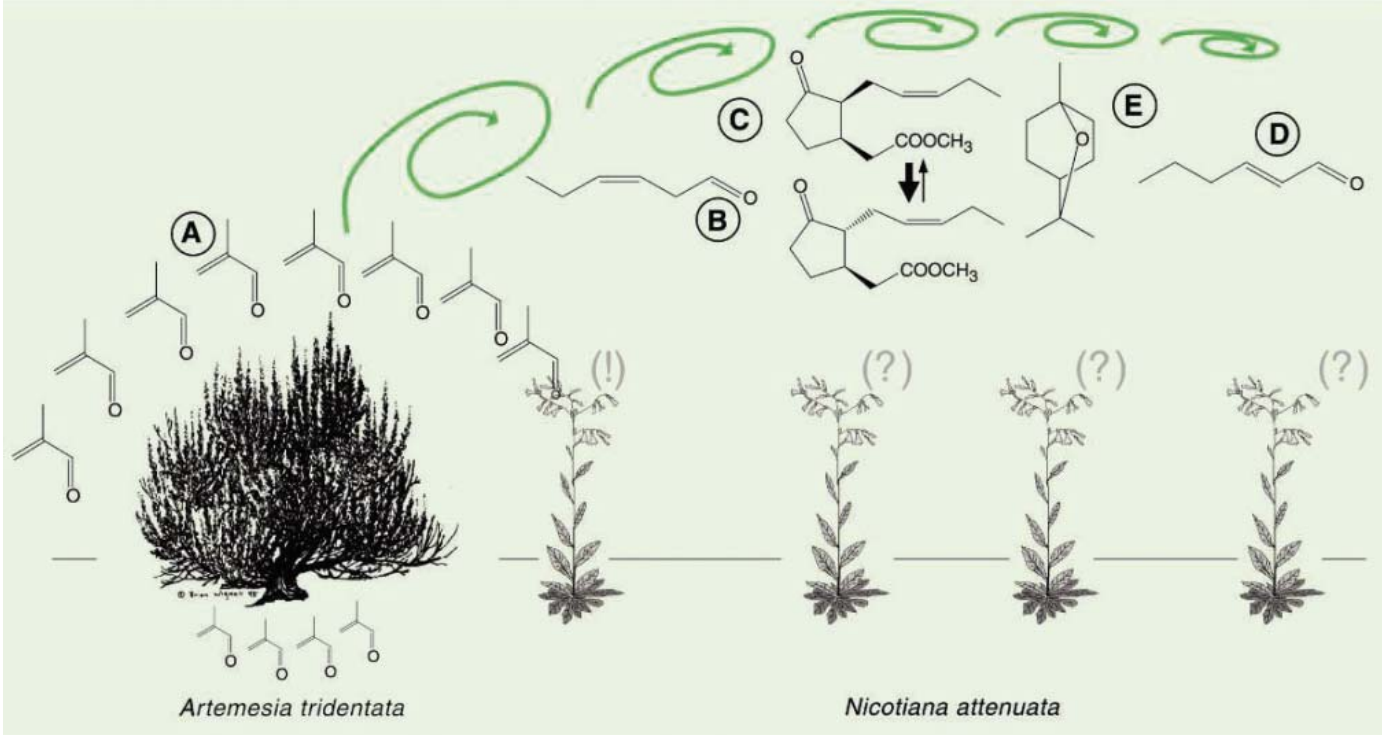

Fig. 2. Aerial interaction of the wild tobacco (*Nicotiana attenuata*) and sagebrush (*Artemisia tridentata tridentata*) (*40*) is the best-documented example of between-plant signaling via above-ground VOCs in nature (*14*, *15*, *41*). When transplanted to within 15 cm of clipped sagebrush, tobacco plants suffered less herbivory and produced more seed capsules than did plants transplanted adjacent to undamaged sagebrush. Damaged sagebrush releases a variety of VOCs, which are composed of highly volatile substances that disperse by diffusion, namely, methacrolein (A) and less volatile compounds such as GLVs [e.g., *cis*-3-hexenal (B) and *trans*-2-hexenal (D)], oxygenated monoterpenes [e.g., cineole (E), thujone, and camphor] and the epimers of MeJA (C), which are likely transported by turbulent flow in fragmented plumes. The plume from damaged sagebrush is highly enriched in the *cis* epimer of MeJA, which is thermodynamically unstable but putatively more biologically active than the *trans* epimer (*14*, *30*, *42*). Hence, MeJA was the most obvious candidate for the volatile signal mediating the response; subsequent studies were unable to confirm that either epimer of MeJA elicited known herbivore defenses when applied in quantities relevant to those released by damaged sagebrush (*30*, *42*). Rather than directly eliciting defenses, exposure to volatiles from excised sagebrush foliage (and two constituents of its aromatic headspace: *trans*-2-hexenal and methacrolein) primes defense responses, so that plants increase the production of their defense protein, PI, faster when attacked (*13*). The progress in this system highlights the difficulty of predicting how plant-plant signaling functions from first principles.

herbivore- or wound-induced vocabularies have been modified by silencing genes involved in either the biosynthesis of particular volatiles or the oxylipin signal cascade represent potential mute emitter plants (Fig. 1).

Mutants whose perception of specific VOCs is impaired ("deaf" plants) represent another tool for analyzing the consequences of VOC signaling as illustrated by the ethylene-insensitive tobacco plants, *etr1-1*. The produce industry long ago developed a sophisticated ethylene trapping and releasing technology, but the first clear demonstration of the functional significance of ethylene signaling in competitive interactions required plants that were "deaf" to this VOC (*20*). Receptors for most of the herbivore-induced VOCs remain to be discovered, but transcriptional responses to VOC exposure can be used in mutant screens to identify new VOC receptors. Identification of these genetic elements and the creation of VOC-reporter plants [with β-glucuronidase (GUS) or green fluorescent protein] will allow researchers to readily determine the quantity of signals that are perceived by receivers at different distances from an emitter. Combining deaf and mute plants with wild-type plants in natural settings will clarify the relevance of VOC signaling for a plant's performance and/or fitness in the real world. Because differences in performance among plants that are unable to produce or perceive certain volatiles are likely to be subtle, the analysis will likely require long-term studies in natural settings. The more deaf plants that are available to complement the growing list of available mute plants, the more tools researchers will have to fully evaluate the significance of volatile signaling among plants in natural settings. These experiments will determine whether being a native speaker enhances a plant's fitness in its community.

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REVIEW

Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value?

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Plants produce many volatile metabolites. A small subset of these compounds is sensed by animals and humans, and the volatile profiles are defining elements of the distinct flavors of individual foods. Flavor volatiles are derived from an array of nutrients, including amino acids, fatty acids, and carotenoids. In tomato, almost all of the important flavor-related volatiles are derived from essential nutrients. The predominance of volatiles derived from essential nutrients and health-promoting compounds suggests that these volatiles provide important information about the nutritional makeup of foods. Evidence supporting a relation between volatile perception and nutrient or health value will be reviewed.

Plants are capable of synthesizing tens to hundreds of thousands of primary and secondary metabolites with diverse biological properties and functions. Plant volatile organic compounds (defined hereafter as volatiles) generated from both primary and secondary metabolites are generally low molecular weight lipophilic compounds (*1*, *2*). More than 7000 flavor volatiles have been identified and cataloged from foods and beverages (*3*, *4*). Many volatiles are produced in plant tissues at specific developmental stages—for example, during flowering, ripening, or maturation. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the "flavor fingerprint" that helps animals and humans recognize appropriate foods and avoid poor or dangerous food choices.

Although perception of flavor is often described as a combination of taste and smell (*5*), appearance, texture, temperature, mouth feel, and past experience also play major roles in flavor perception, indicating that multiple distinct sensory inputs are processed to generate the overall sensation (Fig. 1). Integration of this sensory information in the brain ultimately results in a flavor preference or aversion with a strong influence on subsequent perception and behavior. Studies of flavor preferences and aversions suggest that flavor perception may be linked to the nutritional or health value associated with the perceived foods (*6*–*11*). For example, fatty acids that stimulate taste responses are essential long-chain cis-polyunsaturated fatty acids rather than nonessential saturated fatty acids (*11*). Flavor preferences begin to develop before birth and develop rapidly in the newborn

(*12*–*16*). Several feeding experiences are generally required to develop flavor preferences (*17*, *18*), although flavor aversions are learned much more rapidly (*18*, *19*).

The human genome encodes a few dozen functional taste receptors and several hundred olfactory receptors (*20*, *21*). These sensory receptors have evolved to allow recognition of specific foods and their compositions. Taste receptors monitor five distinct modalities of salty, sweet, sour, bitter, and umami (*22*–*24*). Bitter compounds are sensed by a large family of receptors and are used as a warning of undesirable constituents (*25*). Whereas the taste sensory system provides information on major nutrients such as carbohydrates, proteins, and lipids, the olfactory sensory system and the food volatiles with which they interact provide the basis for the diversity of flavors found in the human diet. To more fully understand the links between flavor preferences, volatiles, and nutrition, we consider the volatile chemicals that contribute to tomato flavor. Tomato is a model for fruit development, and more is known about the chemicals contributing to tomato flavor than for any other fruit or vegetable. Virtually all of the major tomato volatiles can be linked to compounds providing health benefits to humans. In most instances, the link is to essential human nutrients. Frequently these volatiles or their precursors have antimicrobial or other health-promoting activities. Thus, flavor volatiles can be perceived as positive nutritional signals.

The impact of a chemical on flavor perception is determined by both its concentration and the odor threshold (our ability to sense it). When expressed as the log ratio of concentration over odor threshold, the value for compounds present at levels exceeding the threshold is positive. Only a small number of the more than 400 volatiles detected in tomato have a positive impact on the flavor profile. Table 1 lists these volatiles in their approximate order of importance. Odor thresholds of these volatiles vary by as much as six orders of magnitude, and some of the most important volatiles are present in very small quantities.

Volatile emissions have evolved to facilitate seed production and dispersal. In that context, the foundations for the flavors associated with most fruits and vegetables existed before crop domestication. Generally, domestication has had a negative effect on tomato flavor and volatile production. Breeding programs have historically focused on yield, color, shape, and disease resistance. Flavor is a complex, multigenic trait providing unique challenges to breeders and has not been a high priority. Selection for yield, fruit size, and shelf-life characteristics in particular has had unintended negative consequences on fruit flavor. Table 1 lists the concentrations of volatiles emitted by fruits for two different tomatoes: *Lycopersicon esculentum* var. *cerasiforme*, a wild accession, and Flora-Dade, a commercial cultivar released in 1976. Whereas the former is indicative of volatiles produced by the undomesticated species, the latter is typical of most commercial cultivars grown for fresh market consumption. Overall, the sugars, organic acids, and volatiles associated with tomato flavor are somewhat reduced in cultivated varieties (although the yield may be enhanced). A major exception is 6-methyl-5-hepten-2-one, a volatile derived by oxidative cleavage of lycopene. This reflects breeders' emphasis on selection of cultivars with enhanced red color (dependent on lycopene). Another fruit that has been intensively domesticated with similar consequences is strawberry. Cultivated strawberries have different volatile profiles and are considered to be less flavorful than the wild species. A large part of this difference can be attributed to loss of a single enzyme during domestication (*26*).

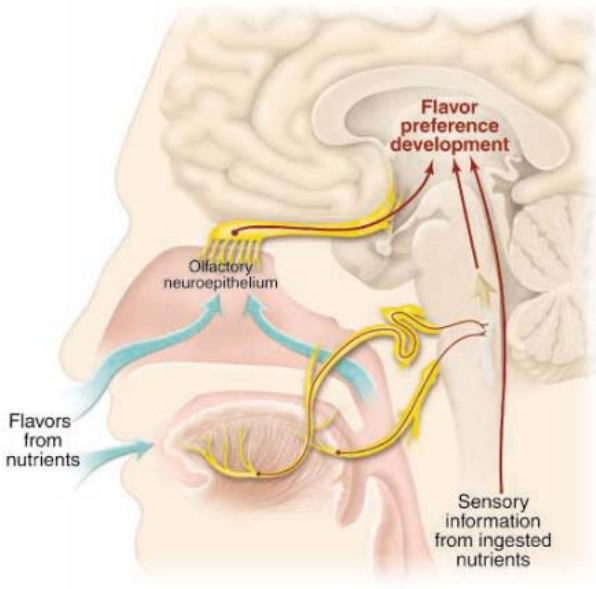

Fig. 1. Taste and olfactory sensory stimulation are integrated with a variety of sensory inputs including visual, tactile, and nutrient-sensing from the gastrointestinal tract to generate the overall flavor perception of specific foods. Experience modulates flavor preferences and aversions.

The metabolic pathways for synthesis of many important plant flavor volatiles are not fully elaborated. However, on the basis of structural considerations, predicted precursor-product correlations, isotope feeding studies, and, in some instances, gene cloning, the precursors of most of the major tomato flavor volatiles are known (Table 1) (*27*). The most abundant volatiles in tomato fruits are derived from catabolism of essential fatty acids (*28*). These volatiles are associated with flavors described as "tomato," "green," or "grassy." They are derived from linoleic acid (hexanal) and linolenic acid (*cis*-3-hexenal, *cis*-3-hexenol, *trans*-2-hexenal) via lipoxygenase activity (*29*) and are, therefore, indicators of the presence of free fatty acids classified as essential to the human diet. The six-carbon aldehydes and alcohols derived from omega-3-linolenic acid are also important constituents of the flavors of a diverse group of plant products including apple, sweet cherry, olive, bay leaf, and tea. Breakdown of linoleic acid generates the decadienoate esters important for pear flavor (*30*), as well as butanoate esters and hexanol that are important for banana flavor (*31*, *32*). Essential fatty acids are also degraded to lactones in peaches, apricots, and coconuts, and many of the fruit aliphatic esters, alcohols, acids, and carbonyls are derived from essential fatty acids.

A second class of volatiles that contribute positively to tomato flavor is derived from the essential amino acids leucine, isoleucine, and phenylalanine (*27*). Thus, these volatiles are indicative of free amino acid content. These volatiles (2- and 3-methylbutanal, 3-methylbutanol, phenylacetaldehyde, 2-phenylethanol, methyl salicylate) are important flavor constituents of many fruits, including strawberries and apples as well as processed foods such as breads, cheeses, wines, and beer. 2- and 3-methylbutanal are also potato flavor volatiles. Methyl salicylate is commonly known as oil of wintergreen and is the methylated derivative of salicylic acid (aspirin), a known anti-inflammatory and analgesic compound. Although some nonessential amino acids are metabolized to volatiles, most notably cysteine being the precursor of allicin, none is considered a major contributor to tomato flavor. Allicin is an important flavor component of garlic and has reported antibacterial and antifungal activities (*33*, *34*).

A third class of tomato volatiles, the apocarotenoids, is derived from oxidative cleavage

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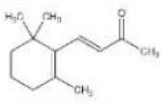
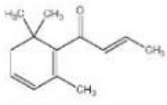
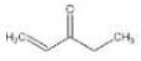
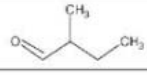
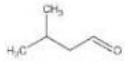
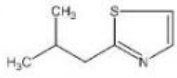
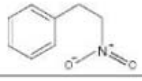
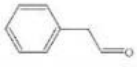
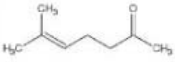
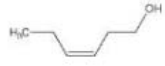
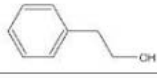
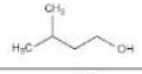
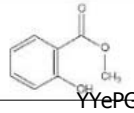
Table 1. Volatile compounds, structures, and their precursors in two varieties of tomato. Shown are volatile chemicals positively contributing to tomato flavor. The rank order of volatiles is based on the work of Buttery and Ling (*27*). The concentrations of volatile emissions were determined for two varieties of tomato. *L. esculentum* var. *cerasiforme* LA1673 is a wild accession isolated by C. Rick in Peru. Flora-Dade is a commercial cultivated tomato released by the University of Florida in 1976. Odor thresholds in parts per billion (ppb) are taken from Leffingwell and Associates (*62*). FW, fresh weight; ND, not determined.

Volatile	Structure	Precursor	Concentration (nl/g FW/hour *cerasiforme*)	Concentration (nl/g FW/hour Flora-Dade)	Odor threshold (ppb)
cis-3-Hexenal		Fatty acid	16.28	5.25	0.25
β-Ionone		Carotenoid	0.03	0.02	0.007
Hexanal		Fatty acid	27.21	17.15	5
β-Damascenone		Carotenoid	ND	ND	0.002
1-Penten-3-one		Fatty acid	0.21	0.03	1
2-Methylbutanal		Isoleucine	0.75	0.25	1
3-Methylbutanal		Leucine	0.67	0.18	0.2
trans-2-Hexenal		Fatty acid	0.7	0.26	17
Isobutylthiazole		Unknown	0.32	0.8	3.5
1-Nitro-2-phenylethane		Phenylalanine	0.018	0.013	2
trans-2-Heptenal		Fatty acid	0.16	0.13	13
Phenylacetaldehyde		Phenylalanine	0.06	0.09	4
6-Methyl-5-hepten-2-one		Carotenoid	0.99	1.84	2000
cis-3-Hexenol		Fatty acid	19.83	13.29	70
2-Phenylethanol		Phenylalanine	0.21	0.32	750
3-Methylbutanol		Leucine	3.83	1.23	120
Methyl salicylate		Phenylalanine	0.08	0.04	40

of carotenoids. Carotenoids are light-harvesting pigments and essential antioxidants in plants. They also provide important visual cues associated with fruit ripeness. Carotenoids have been reported to serve as antioxidants in the human diet and are implicated in many aspects of human health (*35*, *36*), although these benefits remain controversial (*37*, *38*). Nonetheless, the pro–vitamin A carotenoids, principally β-carotene, are essential precursors of retinol, retinal, and retinoic acid. Humans have a much lower odor threshold for β-ionone (the oxidative cleavage product of β-carotene) than for linear carotenoids such as 6-methyl-5-hepten-2-one (derived from lycopene), although both are readily detectable in tomato fruits. Apocarotenoids are important for flavor in diverse food products. For example, β-damascenone, in addition to tomato, is found in berries, apples, and grapes (as well as wine). Safranal, found in saffron, grapefruit, and green tea, is derived from the carotenoid zeaxanthin. Likewise, dihydroactinidiolide and 4-oxoisophorone are flavor components of carotenoid origin found in teas, tobacco, lemon balm, and saffron.

Although a few tomato flavor volatiles are produced in detectable quantities throughout fruit development, most are principally associated with ripening (Fig. 2). Synthesis of the apocarotenoids β-ionone, geranyl acetone, and 6-methyl-5-hepten-2-one increases 10- to 20-fold as fruits reach a fully ripened stage (*27*, *39*). The specific association of these volatiles with ripe fruits and their relative absence from vegetative tissues suggests a role in signaling ripeness and attracting seed-dispersing organisms, including humans. Thus, tomato flavor can be viewed as a set of cues that together reflect the ripeness and nutritional quality/nutrient availability of the fruit. Sweet taste receptors respond to the sugars, principally glucose and fructose that accumulate only upon ripening. Sour taste receptors respond to citrate, malate, and ascorbate. Umami taste receptors respond to the buildup of glutamate and aspartate released from proteins. These signals are integrated with olfactory-system stimulation by volatiles derived from fatty acids, amino acids, and carotenoids. Fruit ripening thus involves a conversion of higher molecular weight precursors to smaller chemical components that provide maximal nourishment to the seed and attraction to seed-dispersing species. Volatiles released during fruit ripening are sensed as principal flavor constituents that signal the ripeness of the fruit and therefore the highest nutrient bioavailability.

Unlike ripening fruits, vegetables produce most of the volatiles sensed as flavors only after their cells are disrupted (*28*). This disruption mixes substrates with the enzymes responsible for generating flavor volatiles. For example, garlic, onions, and mustards, as well as certain other vegetables, produce the volatiles allyl isothiocyanate and allicin after cellular disruption. These volatile flavor compounds exhibit antimicrobial activity when present in a variety of foods. Thioglucosidase activity in various *Brassicaceae* releases volatiles from glucosinolates, which have anticancer activities but can be toxic at high doses. Both the development of flavors and the availability of nutrients are promoted by cell lysis in vegetables.

The volatiles synthesized in popular spices found throughout the world again suggest that flavor perception is linked with specific health properties. Curcumin, a major flavor volatile of the spice turmeric, is reported to have both anti-inflammatory and anti-tumor activities (*40*–*42*). Likewise, curcumene, gingerol, and gingerone from the spice ginger have reported antioxidant and anti-tumor activities. Many spices with distinct preferred flavors in a variety of cultures are reported to have antimicrobial activities, including allicin from garlic, thymol, borneol, isoborneol, eugenol, allyl isothiocyanate, and cavracol from rosemary, sage, clove, mustard, chili pepper, and thyme (*43*–*45*). These observations have led to the proposal that spice use in different parts of the world helps preserve food and provide a safer food supply (*43*). A preference for the flavors found in these spices is believed to have developed due to the health benefit of less contaminated food.

Although bitter taste is generally considered a negative sensation and a warning of toxin content, some bitter flavors are preferred in specific food products. For example, the lupulins from hops, quinine from cinchona, and methyl cinnamate, cineol, and camphor from the spice galangal are preferred bitter flavors in some foods or beverages. Specific health benefits have been reported to be associated with these bitter flavors: Quinine is a well-known antimalarial compound, hops are used as a preservative in beer, and camphor and methyl cinnamate are reported to have antimicrobial activity. Likewise, the bitter gluconsinolates such as sinigrin from brussels sprouts are reported to have anticarcinogenic and immune-boosting activities.

The question of whether a sensory feedback system involving plant-produced volatiles is quantitative or qualitative remains unanswered. Although there is frequently a direct correlation between precursor content and volatile emissions, as with carotenoids (*39*, *46*, *47*), there is not always a direct correlation between essential nutrients and their volatile metabolites. However, this does not exclude a quantitative response because such a response need not be linear. Indeed most biological systems are linear over a limited range. Although excessively high concentrations of many volatiles can be perceived as off-odors, the cues provided by individual volatiles must be considered in the context of the food and the learned experiences associated with that food. Thus, humans respond to a tomato as a whole food with certain nutritional benefits.

Although the physiological mechanisms responsible for monitoring the nutritional or health value of a specific food remain unresolved, behavioral research supports a connection between sensory perception, flavor preferences, and health benefits. For example, rodent feeding studies demonstrate that preferences for bitter or otherwise undesirable flavors can be learned when those flavors are associated with desirable nutrients (*48*). Herbivores learn to consume toxin-containing plants with additional foods that neutralize the toxic effects (*49*–*52*). Even nematodes, with only a few hundred neurons, avoid foods with detrimental health consequences via learned olfactory-mediated responses (*53*). In addition, invertebrate predators forage selectively to acquire specific dietary nutrients (*54*), and grasshoppers feed selectively to maintain dietary protein and lipid content (*55*). Caterpillars regulate their protein and carbohydrate intake (*56*). Tiger moth caterpillars display enhanced taste responses to alkaloid-containing plants when

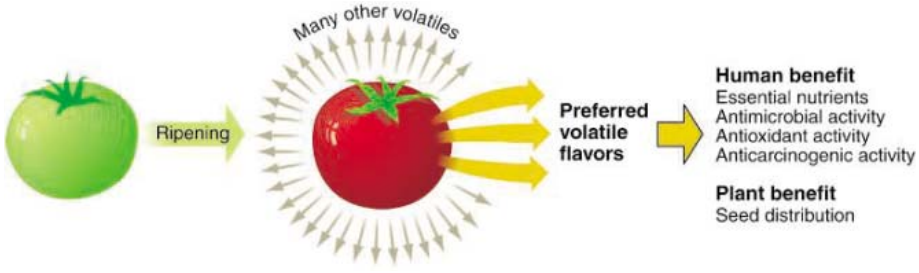


Fig. 2. Tomato fruits produce a volatile emission profile that is both attractive to humans and an indicator of ripeness. Of the more than 400 volatiles emitted by tomato fruits, only a small number, almost all of which are derived from essential human nutrients, are detected and integrated into a preferred volatile aroma. This pattern of volatile emissions is mutually beneficial. Thus, volatile emissions are both positive indicators for the presence in the fruit of compounds with positive health benefits and attractants that promote seed dispersal.

parasitized, resulting in feeding behavior that eliminates the parasites (*57*). Sensory systems expressed throughout the gastrointestinal tract may provide feedback on the quality and quantity of ingested nutrients (*58*–*61*).

In conclusion, a correlation exists between health and the volatiles that contribute to the positive perception of foods. It is likely that volatile emissions have evolved in part to provide positive information to seed-dispersing organisms. For tomato, almost every important volatile is derived from an essential nutrient. Not all desirable volatiles are expected to be derived from essential nutrients, nor will all volatiles derived from essential nutrients be viewed as desirable across all populations. For example, many flavor volatiles are derived from terpenoids that are not directly related to essential nutrients. But many of these terpenoids are also known to have strong antimicrobial activity. Also, nutrients such as essential fatty acids can be metabolized to produce off-flavors in certain circumstances such as the off-flavors generated by lipoxygenase activity in soybean processing. Despite the exceptions, essential nutrient-derived volatile flavors are positive indicators of their precursors. The molecular mechanisms underlying nutrient monitoring remain undiscovered, but implications for food production and consumption are suggested. Much of the developed world faces a nutritional crisis, where obesity and diet-related health issues are becoming an increasing burden to society. Processed foods prevalent in developed countries today often combine natural or synthetic flavors with low nutrient content. Dissociation of flavors from their natural nutritional context may create undesirable health consequences such as the overconsumption of highly processed starch or saturated fats. Flavor preferences together with health benefits should be considered in future food production and in crop-enhancement strategies.

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The Age of the Sahara Desert

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After the mid-Holocene humid period (~6000 years ago), arid conditions developed throughout North Africa, culminating in the formation of the Sahara, which is the largest warm-climate desert on Earth (~9,000,000 km^2). However, earlier desert recurrences in the region are also documented. Direct evidence for eolian deposition is given by thermoluminescence dating for the Late Pleistocene; e.g., in Mauritania [25 to 15 thousand years ago (ka)] (*1*) or in Tunisia (86 ka) (*2*). The latter is currently considered as the oldest terrestrial record for desert conditions in the Sahara (*2*), even if firm evidence exists for a pre-Quaternary Great Western Sand Sea in Algeria (*3*). Some earlier arid episodes (Miocene-Pliocene) were also suggested by marine records off West Africa (*4*); but until now, no contemporary in situ eolian deposits were known in the Sahara region. In the northern Chad Basin, we recently identified and dated widespread outcrops of eolian dune deposits that are distributed over an area more than 2000 km^2. Our results testify that the onset of recurrent desert conditions in the Sahara started at least 7 million years ago (*5*–*7*).

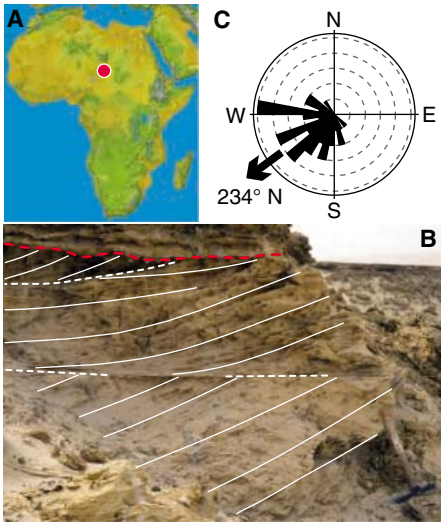

Fig. 1. (**A**) Location of the study area (red dot). (**B**) One example of the eolian sandstones in the Djurab area (16°13′42″N, 17°32′23″E) showing three cross-stratified sets (white lines) conformably overlain by the Upper Miocene fossils-rich perilacustrine sandstones (contact: red dotted line). The hammer is shown for scale (45 cm). (**C**) Paleowinds as shown in a rose diagram including 39 measured dune foresets [same locality as in (B)]. The black arrow indicates the mean resultant direction (234°N, 95% confidence interval = ±16°).

In interdune areas of the Djurab sand sea (Northern Chad, 16° to 16°20′N, 17° to 19°E) (Fig. 1A), pre-Quaternary successions show sandstone horizons alternating with lacustrine mudstones and diatomites. In the Central Djurab (16°15′N, 17°30′E), sandstone outcrops made up of poorly cemented quartz arenites are characterized by very well rounded sand grains with frosted surfaces. Large-scale thinning-up (2 to 0.2 m thick) successions of highly dipping (angle of 30° ± 2°) cross-strata are ubiquitous (Fig. 1B). In horizontal surfaces, present-day deflation highlights well-developed festoons that are up to 20 m wide. Cross-strata comprise alternating coarse-grained and fine-grained laminae, displaying a well-sorted bimodal grain size distribution. Texture and sedimentary structures indicate that these sandstones were deposited by migrating eolian sand dunes with typical grain flow and grain fall laminae preserved in foresets. Consistent dips in cross-strata reflect dominant paleowind orientation from the east-northeast to the west-southwest (Fig. 1C). Wind ripples, with similarly oriented axis, are occasionally preserved at the base of some foresets, recording secondary airflow along the dune lee faces. In the Toros Menalla region, these eolian sandstones are conformably overlain by a horizon bearing abundant vertebrates fossils, including *Sahelanthropus tchadensis*, the earliest known Hominid (*5*, *7*). In this horizon, named the Anthracotheriid Unit, biostratigraphic correlation of the mammalian fauna indicates an age of 7 Ma (*5*–*7*).

Additionally, eolian horizons are also recorded in the eastern Djurab (16°19′N, 18°41′E). Here, 0.5-m-high and 2-m-long basal dune foresets, dipping toward the west-southwest, are well developed. As for the previous locations, mammalian faunas within a conformably overlying sandstone indicate an age of 5 to 5.5 Ma (*8*). Moreover, for slightly younger layers (4 to 4.5 Ma), episodic arid conditions are also testified in nearby areas (16°20′N, 18°58′E) by eolian deposits and by fossil termite nests attributed to arid-environment–limited species (*9*, *10*).

The northern Chad Basin eolian deposits provide the earliest (7 Ma, Upper Miocene) in situ record for arid climate and eolian sand accumulation in the Sahara. East-northeast–west-southwest winds (trade winds) prevailed in this region then, as they do today. This geological record extends considerably the demonstrated age for the onset of desert conditions in the Sahara. Repeated eolian-lacustrine sequences are strong evidence for arid-humid climate changes, suggesting that desert conditions existed repetitively rather than continuously, as in the Quaternary. Maley (*11*) showed that the length of a similar sequence in the Late Quaternary of Chad Basin was ~20 thousand years and could so be associated with Milankovitch cycles. In addition, this terrestrial record validates distal marine proxies of pre-Quaternary Saharan dry episodes. In the future, paleoclimate models should integrate the age of strong arid recurrences over the Sahara at the scale of several millions of years. The creation of an eolian rock record is considered to be a very selective process (*12*), as confirmed by the rarity of preserved eolian deposits in North Africa. Chad Basin appears, therefore, as a unique place in the Sahara where, even if truncated, ancient eolian deposits are preserved, supplying thus the current lack of terrestrial records for the Miocene-Pliocene arid climate episodes in the Sahara.

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13. We thank M. Deynoux, S. Kröpelin, J. Maley, G. Ramstein, C. Roquin, P. Sepulchre, J.-J. Tiercelin, and D. Verschuren for discussions; P. Huntsman-Mapila for manuscript reading; and three anonymous reviewers for contributing to the improvement of this work. We also thank the Chadian Authorities [Ministère de l'Education Nationale de l'Enseignement Supérieur et de la Recherche; Université de N'Djaména; Le Centre National d'Appui à la Recherche (CNAR); and B. El-Hadj Mallah]; the Ministère Français de l'Education Nationale (Faculté des Sciences, Université de Poitiers); Ministère de la Recherche (CNRS: Département SDV and ECLIPSE); Ministère des Affaires Etrangères (DCSUR, Paris and SCAC, N'Djamena); the Région Poitou-Charentes; the NSF program Revealing Hominids Origin Initiative (to F. C. Howell and T. D. White); and the Armée Française (MAM and Epervier) for logistical support. We thank all the other Mission Paléoanthropologique Franco-Tchadienne members who joined us for field missions; S. Riffaut and X. Valentin for technical support; and G. Florent and C. Noël for administrative MPFT guidance. Paleowinds data have been plotted with GEOrient (free software by R. Holcombe; available at www.holcombe.net.au/software/index.html). This work is contribution N°981 of the European Institute for Marine Studies (IUEM) (Brest, France).

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The Ste5 Scaffold Allosterically Modulates Signaling Output of the Yeast Mating Pathway

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Scaffold proteins organize signaling proteins into pathways and are often viewed as passive assembly platforms. We found that the Ste5 scaffold has a more active role in the yeast mating pathway: A fragment of Ste5 allosterically activated autophosphorylation of the mitogen-activated protein kinase Fus3. The resulting form of Fus3 is partially active—it is phosphorylated on only one of two key residues in the activation loop. Unexpectedly, at a systems level, autoactivated Fus3 appears to have a negative regulatory role, promoting Ste5 phosphorylation and a decrease in pathway transcriptional output. Thus, scaffolds not only direct basic pathway connectivity but can precisely tune quantitative pathway input-output properties.

Cells use networks of intracellular signaling proteins to detect and process environmental stimuli and to make complex response decisions. A central question in cell biology is how such signals are accurately and specifically transmitted through these pathways, especially given the vast number of similar signaling proteins that exist in a given cell. In many cases, scaffold proteins—proteins that bind and organize multiple proteins within a pathway—have emerged as important factors in mediating signaling efficiency and specificity (*1*, *2*). By tethering components together, scaffolds are thought to promote interaction of the proper partners and to prevent signaling to improper partners. The scaffold protein Ste5 is required for signaling through the mating (or pheromone) response mitogen-activated protein kinase (MAPK) pathway in *Saccharomyces cerevisiae* (*3*). Ste5 has separable binding sites for each member of the mating MAPK cascade: the MAPK Fus3, the MAPK kinase (MAPKK) Ste7, and the MAPKK kinase (MAPKKK) Ste11 (*4*–*6*). A scaffold is thought to be particularly important for directing signals through the mating pathway, because several functionally distinct MAPK cascades in yeast use an overlapping set of kinase components (e.g., Ste11 is also a member of the osmoresponse and filamentation pathways, and Ste7 is also a member of the filamentation pathway) (*7*, *8*).

Despite the importance of Ste5 as a prototypical scaffold, little is known about the structural and molecular basis for its function (*1*, *9*, *10*). How does it interact with the kinases and how does it promote proper signaling? Here, we focus on understanding how the mating MAPK, Fus3, is recruited to the Ste5 complex. We mapped the interaction sites, determined the structural basis of the interactions, and analyzed how they contribute to pathway signaling in vivo. We uncovered several unexpected findings: Within the Ste5 complex, multiple independent recruitment sites for Fus3 contribute to pathway function; some of these sites do not function as passive tethering sites but rather can allosterically activate the kinase; and these sites can precisely modulate pathway output, not only by promoting signal propagation but also by mediating phosphorylation events that limit pathway output.

Mapping Fus3 binding sites. The MAPK Fus3 physically interacts with two members of the mating pathway, the scaffold Ste5 and the upstream MAPKK Ste7 (Fig. 1A). Fus3 interacts with Ste7 through a canonical MAPK docking interaction; Ste7 contains a motif matching the consensus sequence $(R/K)_{1,2}x_{3-8}LxL$ (one or two Arg or Lys in the first positions, a spacer region three to eight amino acids in length, and two Leu residues separated by one amino acid) (*11*, *12*). Such docking motifs are found in diverse MAPK binding partners and bind to a groove on the surface directly opposite the kinase active site (*13*–*15*). Previous studies have suggested that the docking motif in Ste7 makes a marginal contribution to mating pathway function (*12*). However, we have recently found that Ste7 contains a second MAPK docking motif, also near the N terminus (*16*) (Fig. 1B), and we present a functional analysis of both motifs here. We have also solved the structure of one of the Ste7 docking peptides bound to Fus3 (*16*).

In contrast, little is known about the interaction of Fus3 with Ste5. This interaction was first mapped by yeast two-hybrid analysis to a 96–amino acid stretch in Ste5 (*4*). We refined this binding region through a series of deletion constructs to a minimal ~30-residue polypeptide [residues 288 to 316 (Ste5_pep)] that is sufficient for binding (Fig. 1B and fig. S1). This polypeptide shows no apparent similarity to other MAPK docking motifs, including the docking peptides in Ste7. Using fluorescence polarization, we have measured the dissociation constant (K_d) of this Ste5 fragment for Fus3 to be 4 μM (fig. S2), which is comparable to the affinities of the docking peptides from Ste7 ($K_d^{Ste7\_pep1} = 0.08$ μM; $K_d^{Ste7\_pep2} =$ 12 μM) (*16*).

Structure of Fus3-Ste5 complex and comparison to canonical MAPK docking interactions. We solved the crystal structure of the Ste5 fragment in complex with Fus3 (Fig. 2) (*17*). This complex is unlike others observed for MAPKs. The Ste5 fragment binds Fus3 in a bipartite manner, extending over the entire backside of the kinase to contact

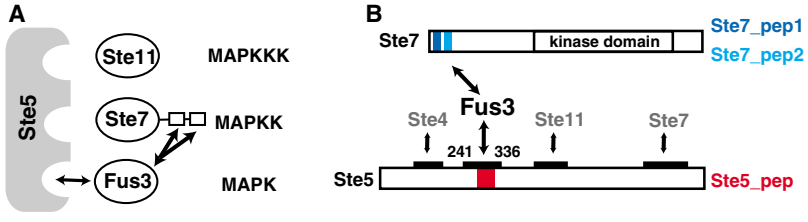

Fig. 1. Fus3 recruitment to the pheromone response MAPK complex. (**A**) Schematic of pheromone response MAPK complex. The MAPK Fus3 interacts with the scaffold protein Ste5 (*4*–*6*) and the MAPKK Ste7 (*6*, *40*). (**B**) Maps of the interaction domains in the MAPKK Ste7 and the scaffold Ste5. Minimal Fus3 binding peptides are shown in color [dark blue, Ste7_pep1 (*12*, *16*); light blue, Ste7_pep2 (*16*)]. Black bars above the Ste5 schematic indicate protein-interaction domains identified in yeast two-hybrid assays (*4*, *37*). The Fus3 binding peptide (Ste5_pep) is shown in red (fig. S1).

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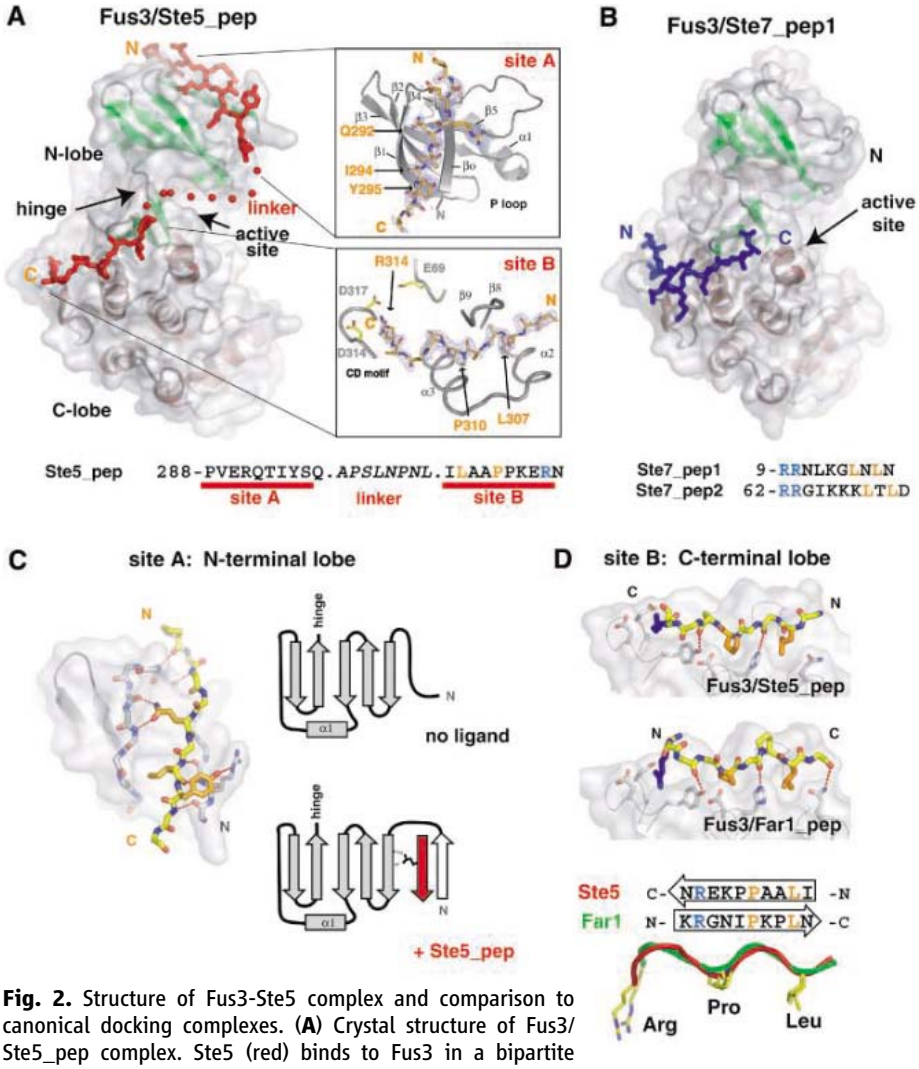

Fig. 2. Structure of Fus3-Ste5 complex and comparison to canonical docking complexes. (**A**) Crystal structure of Fus3/Ste5_pep complex. Ste5 (red) binds to Fus3 in a bipartite manner. Close-up views of site A and site B on the right are shown with simulated annealed electron density omit maps (contoured at 1σ) for the Ste5 peptide. (**B**) Structure of Fus3 in complex with a canonical docking motif from Ste7 (Ste7_pep1) (*16*). (**C**) Protein-protein interactions at site A. The N-terminal half of Ste5_pep adopts a β-strand conformation and initiates the formation of a new β strand at the N terminus of Fus3 (β0). This strand forms eight backbone-backbone H bonds with the Fus3 N-terminal region (H bonds are indicated with red dashed lines). The side chain of Q^{292} is H bonded to the backbone of β1, the hydrophobic side chain of I^{294} interacts with a groove on the top of the kinase, and Y^{295} makes an H bond with the side chain of R^4 from Fus3. Schematic illustration of secondary structural elements of the N-terminal kinase lobe in the unliganded and Ste5_pep liganded complex is shown on the right. (**D**) Comparison of protein-protein interactions at the canonical MAPK docking groove (site B) between the Fus3/Ste5_pep and the Fus3/Far1_pep complexes (*16*).

two distinct surfaces. The N-terminal portion of the Ste5 fragment contacts the N-terminal lobe of the kinase (site A), and the C-terminal portion contacts the C-terminal lobe of the kinase (site B). The intervening linker region of eight residues between site A and site B binding motifs is disordered and not visible within the crystal structure. Binding of Ste5_pep buries ~1000 Å^2 of surface area with a roughly even contribution from the A and B sites. Neither the A nor the B site fragment from Ste5 independently shows measurable binding to Fus3 (*18*).

The B site interaction in the Ste5-Fus3 complex overlaps with the binding surface of the kinase that interacts with canonical docking motifs, such as those found in Ste7. This explains why interaction of Fus3 with Ste7 and Ste5 is competitive (*19*) (Fig. 2, A and B). The nature of the interaction, however, is quite different; the Ste5 fragment lies in the docking groove in an N- to C-terminal orientation that is precisely the opposite of that of the canonical docking peptides. Despite this difference in orientation, the B site interaction bears some similarities to canonical docking interactions, particularly a recently solved complex of Fus3 with a docking peptide from the substrate Far1 (*16*). Although the peptides bind in opposite orientations, both insert a proline into a central pocket in the Fus3 surface and present a peripheral Arg that forms electrostatic interactions with a conserved pair of Asp residues (Fig. 2D). The backbone trace of these two peptides, although reversed, is virtually identical, as are many of the hydrogen bonds made to the peptide backbone. The flexibility of the Fus3 binding site to recognize peptides in two orientations is reminiscent of the properties of Src homology 3 (SH3) domains and other domains that recognize proline-rich peptides in two possible orientations. In the case of Fus3, both the Far1 and Ste5 peptides, in their central regions, adopt a polyproline II (PPII) helical conformation. PPII helices are twofold rotationally pseudosymmetric; thus, any protein designed to bind this structure will inherently have some ability to recognize peptides in a reverse orientation (*20*). This recognition flexibility of the MAPK docking groove indicates that there may be additional classes of MAPK interacting motifs that have not yet been identified.

The interactions at the A site have no obvious similarity to previously characterized kinase-peptide interactions. This region of the kinase N-terminal lobe normally forms a five-stranded β sheet. However, upon binding, the Ste5 peptide itself forms a β strand and induces Fus3 residues 5 to 10 to form a sixth β strand, and the region adopts a seven-stranded structure in the form of a β sandwich (Fig. 2C).

Ste5 allosterically activates Fus3 autophosphorylation. The Ste5 fragment not only binds Fus3 in a noncanonical manner but also allosterically stimulates the rate of Fus3 autophosphorylation by ~50-fold (Fig. 3A). Such strong activation is not observed with any other known Fus3 binding peptides, including the docking motifs from Ste7 (Fig. 3B). Mass spectrometric and mutational analysis indicates that Ste5 stimulation produces a monophosphorylated form of the kinase: Autophosphorylation occurs selectively on Tyr^{182}, one of two residues (Thr^{180} and Tyr^{182}) in the Fus3 activation loop that are normally phosphorylated upon full activation of the MAPK (fig. S3) (*21*). Monophosphorylation (pTyr) substantially increases kinase activity; with the myelin basic protein as a model MAPK substrate, the ratio of activity of the nonphosphorylated, tyrosine-phosphorylated, and doubly phosphorylated forms of Fus3 is 1:25:120 (fig. S4). Thus, unlike other MAP kinases, such as Erk2 (*22*), the monophosphorylated form of Fus3 is active in vitro, though it is still activated another four- to fivefold when doubly phosphorylated.

We solved the structure of the pTyr form of Fus3 (fig. S5). Comparison with the nonphosphorylated form of the kinase provides a model for why the pTyr form shows relatively high activity. Before phosphorylation, part of the activation loop occludes the active site, acting as a pseudosubstrate (*16*). However, in the pTyr form, the entire activation loop is disordered and no longer blocks substrate accessibility, which likely accounts for the increased kinase activity. The role of the second phosphorylation (pThr) cannot be directly inferred from available Fus3 structures. However, phosphorylation on Thr^{180} may stabilize a new conformation of the dislodged activation loop by promoting new interactions with the rest of the kinase, as is observed for the structurally similar mammalian MAPK Erk2 (*23*).

The allosteric activation of Fus3 by Ste5 is reminiscent of the enhanced autoactivation of mammalian p38α induced by transforming growth factor β–activated protein kinase 1–binding protein 1 (TAB1) (*24*), although this event leads to dual phosphorylation of p38α rather than monophosphorylation observed for Fus3. Little is known about the mechanistic basis for TAB1-enhanced p38α autophosphorylation.

Mechanism of allosteric activation. How, mechanistically, might the Ste5 polypeptide induce autophosphorylation, and therefore activation, of Fus3? Several pieces of evidence support a model in which the linkage between sites A and B of the Ste5-Fus3 interaction is critical for activation. First, an alignment of peptide-bound and unbound structures observed within the same crystal form (Fig. 3C) reveals that Ste5 binding to Fus3 results in a perturbation of the relative orientation of the N- and C-terminal lobes of the kinase upon Ste5 binding. If such an interdomain hinge motion is important for Fus3 activation, then altering the length of the linker between the site A and site B binding motifs might influence autoactivation. Indeed, lengthening or shortening this linker region by one, two, or three residues reduced the ability of Ste5_pep to enhance Fus3 auto-activation without affecting binding affinity for the kinase (Fig. 3D and fig. S6). Thus, we propose a model in which the Ste5 polypeptide binds to both domains of Fus3, inducing a subtle hinge-bending shift. The shift between the kinase domains may increase the flexibility of the activation loop (*25*), allowing the Tyr side chain to enter the active site, where it can be autophosphorylated (the rate of autophosphorylation is independent of enzyme concentration, consistent with an intramolecular reaction) (*18*).

The overall topology with which the Ste5 peptide interacts with Fus3 is somewhat similar to the way in which the C-terminal extension of protein kinase A (PKA) packs against the main kinase domain (Fig. 3E) (*26*). This C-terminal extension is thought to have an important role in placing the PKA catalytic domain in a constitutively active conformation, perhaps by orienting the two lobes of the kinase in the correct juxtaposition for catalysis (*27*). In both cases, peptide elements, either inter- or intramolecular, that properly position the two kinase lobes with respect to one another may play an important role in activation.

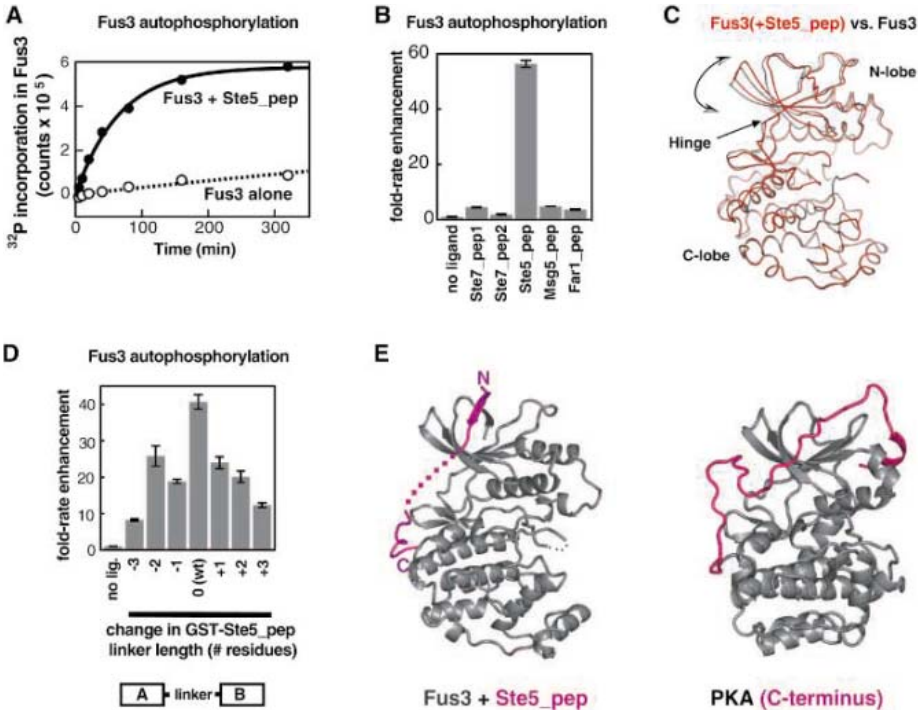

Fig. 3. Ste5 allosterically activates Fus3 autophosphorylation. (**A**) Ste5_pep enhances Fus3 autophosphorylation. Fus3 was incubated with no ligand (open circles) or Ste5_pep (closed circles), and data from autoradiograms were fit to an equation describing unimolecular autophosphorylation kinetics. (**B**) No other Fus3 binding peptides strongly promote autophosphorylation. Autophosphorylation rate enhancements (relative to Fus3 activity alone) are plotted. Msg5 is a phosphatase that acts on Fus3, and Far1 is a Fus3 substrate (*16*). Error bars show 1 SD from a kinetic fit of data averaged from three experiments. (**C**) Comparison of Fus3 with and without Ste5_pep in the same crystal form. (**D**) Effect of lengthening or shortening the linker between the two regions by which Ste5_pep contacts Fus3 (sequence deletions or insertions are listed in table S1). Rate enhancement factors were obtained by measuring ^{32}P incorporation into Fus3 in the presence of each glutathione *S*-transferase (GST)–peptide compared with that with GST alone (fig. S6). Error bars show 1 SD derived from a kinetic fit of data from a typical experiment. (**E**) Comparison of Ste5_pep in complex with Fus3 (left) and the C-terminal tail (amino acids 301 to 350) of the catalytic subunit of PKA (right) (*26*).

In vivo analysis: Ste7 docking sites are redundant but essential for pathway signaling. We biochemically characterized three binding sites for Fus3 within the mating signaling complex. To determine the physiological role of these recruitment sites in the mating response, we made mutant alleles of Ste7 (*16*) or Ste5 in which each of these MAPK recruitment sites was disrupted [nondocking (ND) mutations include disruption of Ste7 docking sites $STE7^{ND1}$, $STE7^{ND2}$, and $STE7^{ND1,2}$, and disruption of Ste5 docking site $STE5^{ND}$), and we quantitatively measured their ability to replace the wild-type gene in vivo (Fig. 4). Mating response to increasing α factor was measured by a mating reporter gene [Fus1–green fluorescent protein (GFP)]. Average pathway output per cell was quantitated by flow cytometry (*17*).

Mutation of either individual Ste7 docking motif reduced maximal pathway output, though output was still clearly detectable (Fig. 4A). However, if both sites were simultaneously mutated ($STE7^{ND1,2}$), no pathway output was observed. Similar results were observed by assaying Fus3 phosphorylation and quantitative mating efficiency (*18*). The effect of disrupting both Ste7 docking peptides is similar to that of disrupting the Ste5-Ste7 interaction (*28*) and approaches that of deleting Ste7. Hence, it appears that the Fus3 docking sites in Ste7 are essential for pathway signaling, although they are functionally redundant.

In vivo analysis: Allosteric activation site in Ste5 down-regulates pathway output. We expected that the region of Ste5 that bound

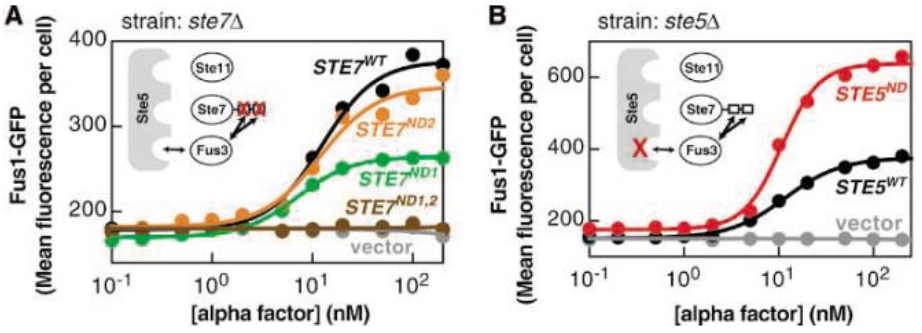

Fig. 4. Negative effects of the Ste5 Fus3 binding region on transcriptional mating response. (**A**) Effects of mutating Ste7 Fus3 interaction sites on the transcriptional mating pathway–dependent reporter Fus1-GFP. Multiple mutations were made in key basic and hydrophobic residues in Ste7_pep1 (allele *STE7^{ND1}*) or Ste7_pep2 (allele *STE7^{ND2}*) to fully disrupt Fus3 binding. GFP expression driven by the pheromone-inducible Fus1 promoter was measured by flow cytometry in yeast expressing the indicated allele of *STE7* in a *ste7*Δ strain (gray, empty vector; black, *STE7^{WT}*; green, *STE7^{ND1}*; orange, *STE7^{ND2}*; brown, *STE7^{ND1,ND2}*). (**B**) Effect of mutating the Fus3 interaction site on Ste5 on expression of the Fus1-GFP reporter. Multiple mutations were made in key residues of Ste5_pep (allele *STE5ND*) to fully disrupt Fus3 binding (fig. S8; mutations are listed in table S1). Experiments were done in yeast expressing the indicated allele of *STE5* in a *ste5*Δ strain (gray, empty vector; black, *STE5^{WT}*; red, *STE5ND*). See fig. S7 for histograms from flow cytometry studies.

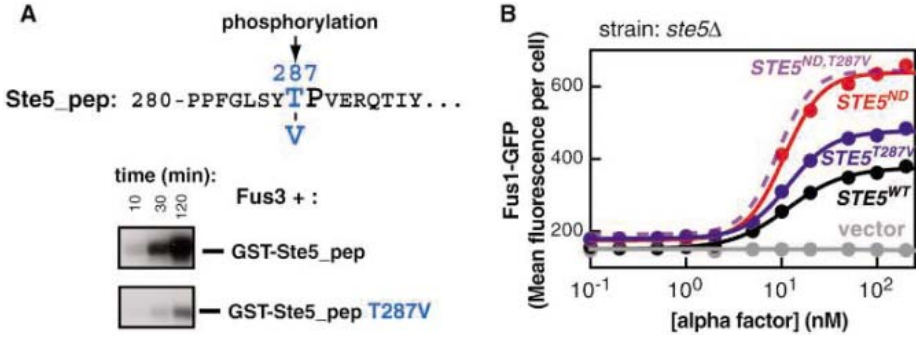

Fig. 5. Importance of phosphorylation of Ste5 in controlling amplitude of pathway output. (**A**) Phosphorylation by Fus3 of an extended version of the Ste5_pep peptide (amino acids 280 to 321) in vitro. Consensus MAPK phosphorylation sequence (S/T-P) is shown in large type, with putative phosphoacceptor Thr^{287} shown in blue. Lower panel shows autoradiogram of ^{32}P incorporation into GST fusions of either the extended Ste5_pep or a T287V mutant of the extended peptide, after incubation with Fus3. (**B**) Effect of mutating this phosphoacceptor residue in Ste5 on expression of the Fus1-GFP reporter. The mutation was made in an otherwise wild-type context (allele *STE5^{T287V}*) or in a nondocking allele of Ste5 (allele *STE5^{ND,T287V}*). GFP expression was measured by flow cytometry in yeast expressing the indicated allele of *STE5* in a *ste5*Δ strain (gray, empty vector; black, *STE5^{WT}*; red, *STE5ND*; blue, *STE5^{T287V}*; fit shown in dashed purple line, *STE5^{ND,T287V}*; data points omitted for clarity).

Fus3 would also make an important contribution to increasing pathway output. Surprisingly, we observed the opposite effect (Fig. 4B and fig. S7). We disrupted the Ste5-Fus3 interaction by mutating six residues distributed through the site A and site B interaction motifs to Ala. [We confirmed that these mutations yield a fragment that can neither bind nor activate Fus3 (fig. S8).] When we replaced wild-type Ste5 with this nonbinding mutant in vivo, we observed a twofold increase in maximal pathway transcriptional output (Fus1-GFP expression). This increase in output level is greater than that which has been observed with most gain-of-function mutants, such as overexpression or constitutive alleles of pathway members (*29*–*31*). The transcriptional difference observed with this Ste5 allele is dependent on Fus3, consistent with our observation that the semi-redundant filamentation MAPK, Kss1, does not bind this fragment (*18*). This mutant phenotype suggests that the normal role of the Fus3-binding region in Ste5, with its unusual ability to enhance Fus3 autophosphorylation, is actually to attenuate pathway output.

Thus, contrary to previous simple models, this particular scaffold-MAPK recruitment interaction appears not to promote signaling; rather, it appears to down-regulate pathway output. In contrast, the MAPKK-MAPK interaction, though redundant, is essential for signaling.

Monophosphorylated Fus3 phosphorylates Ste5 as part of the down-regulatory response. When Ste5 was used to stimulate autophosphorylation of Fus3 in vitro, we noticed that the Ste5 fragment itself became strongly phosphorylated. The Fus3 binding region of Ste5 contains one potential MAPK phosphorylation site (Thr^{287}-Pro^{288}) (Fig. 5A). The Ste5 polypeptide phosphorylation is greatly reduced when this site is mutated [Thr^{287}→Val (T287V)], indicating that this is the primary phosphoacceptor in vitro. Neither phosphorylation of this site nor mutation to Val affects the ability of the polypeptide to bind to and stimulate autoactivation of Fus3 (fig. S9).

Nonetheless, we hypothesized that this phosphorylation of Ste5 might affect pathway down-regulation in vivo, particularly given that feedback phosphorylation occurs elsewhere in the mating pathway (*32*–*34*) and other MAPK pathways (*35*). To test this model, we examined the effect of replacing wild-type Ste5 with a version bearing the T287V mutation (Fig. 5B). This mutant exhibits increased Fus1-GFP output, partially phenocopying the *STE5ND* mutant that prevents Fus3 binding and auto-activation. The *STE5ND* mutation was also epistatic to the *STE5^{T287V}* mutation; although both mutations individually increased pathway output, a version of Ste5 bearing both mutations showed the same maximal transcriptional output as that observed with the *STE5ND* allele. These findings are consistent with a model in which the mutations affect different steps within the same pathway. Thus, we propose that Fus3, when autoactivated by this fragment of Ste5, may promote increased phosphorylation of Ste5 on Thr^{287}.

The precise mechanism by which autoactivation and consequent scaffold phosphorylation down-regulates pathway transcriptional output remains unclear. Phosphorylation of Ste5 might alter turnover and lower steady-state abundance of Ste5 through degradation; it might alter the trafficking properties of Ste5 (*31*, *36*); or this Ste5 phosphorylation event might exert its effects through multiple composite actions. Monophosphorylated Fus3 may also have substrates besides Ste5 that contribute to pathway down-regulation, because the *STE5^{T287V}* allele only partially phenocopies the *STE5ND* allele. The monophosphorylated form of Fus3 may act on distinct substrates from those modified by the dual-phosphorylated, fully active form of the kinase. Alternatively, the mono- and dual-phosphorylated forms of Fus3 might be differentially localized.

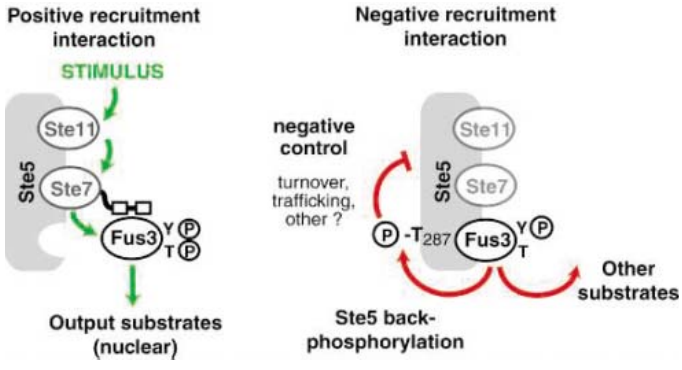

Fig. 6. Schematic of the distinct roles for Fus3 binding peptides in the MAPKK Ste7 and the scaffold Ste5. (**Left**) Two redundant peptides in the MAPKK Ste7 that recruit Fus3 are essential for signaling through the pheromone response MAPK pathway. (**Right**) The Fus3 binding site in the Ste5 scaffold limits signal propagation through the MAPK pathway. Recruitment of Fus3 to this site enhances autophosphorylation on Tyr^{182} of Fus3, which may promote Fus3 phosphorylation of other substrates, including Thr^{287} of Ste5. The net effect of these phosphorylation events appears to be a decrease in transcriptional output of the pheromone response MAPK pathway.

Conclusions: Ste5 scaffold shapes quantitative pathway output. We characterized multiple distinct modes of recruitment of the MAPK Fus3 to the yeast pheromone response pathway signaling complex, a prototypical scaffolded MAPK cascade (Fig. 6). The interaction of the MAPK with the MAPKK Ste7 is required for efficient signal propagation. In contrast, the interaction of the MAPK with the scaffold appears to control pathway gain by down-regulating overall output. Thus, the Ste5 scaffold not only functions as an interaction assembly point for the pathway components, but it also serves as a regulatory node that actively participates in tuning pathway flux.

These findings force us to revise models for how Ste5 and its interactions contribute to pathway function, but they do not contradict the fundamental concept that assembly of the MAPK pathway components into a single complex is important for determining the basic wiring of the pathway. Recruitment of Fus3 to the complex is clearly essential for proper signaling, although this recruitment is primarily mediated through interactions with the upstream MAPKK Ste7. Recruitment of other cascade members (MAPKKK Ste11 and MAPKK Ste7) to Ste5 is also essential for signaling (*28*, *37*). However, the Ste5-Fus3 recruitment interaction studied here, which was previously thought to be essential for signaling, actually limits transcriptional pathway output at a systems level. These findings suggest that multiple molecules of Fus3, some playing positive and others playing negative roles, may be part of an individual signaling assembly.

A model in which Fus3 has both positive and negative regulatory roles is reminiscent of the behavior of transcriptional regulators. Promoters, like scaffolds, organize the assembly of transcription factor complexes that determine the degree of gene expression. There are growing examples in which the same transcription factor can play either positive or negative regulatory roles, depending on the exact context of promoter sequence and other cofactors (*38*, *39*).

This work presents evidence of a more complex role for the scaffold Ste5 in regulating the yeast pheromone response pathway. Rather than merely recruiting catalytic components, the scaffold alters the catalytic activity of at least one bound kinase and takes part in a negative regulatory loop that appears to decrease output from the pathway. Other scaffolds may also have multiple roles in shaping signaling responses, including wiring together specific sets of signaling components and controlling and coordinating their behavior to precisely tune the amplitude and dynamics of the response.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S9
Tables S1 to S3
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Cosmological Magnetic Field: A Fossil of Density Perturbations in the Early Universe

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The origin of the substantial magnetic fields that are found in galaxies and on even larger scales, such as in clusters of galaxies, is yet unclear. If the second-order couplings between photons and electrons are considered, then cosmological density fluctuations, which explain the large-scale structure of the universe, can also produce magnetic fields on cosmological scales before the epoch of recombination. By evaluating the power spectrum of these cosmological magnetic fields on a range of scales, we show here that magnetic fields of $10^{-18.1}$ gauss are generated at a 1-megaparsec scale and can be even stronger at smaller scales ($10^{-14.1}$ gauss at 10 kiloparsecs). These fields are large enough to seed magnetic fields in galaxies and may therefore have affected primordial star formation in the early universe.

Conventional models for the generation of large-scale magnetic fields are mostly classified into two categories: astrophysical and cosmological mechanisms. Astrophysical mechanisms—often involving the Biermann battery effect, in which magnetic fields are generated from an electric current driven by the rotation of the system (*1*)—can explain the small-scale amplification of fields, such as in stars or in supernova explosions (*2*, *3*). However, these mechanisms do not fully explain fields on larger cosmological scales, such as those known to exist in galaxies and clusters of galaxies. Reconnecting the magnetic field lines might increase the coherence length from the size of stars to that of galaxies (tens of kpc), although there is still no convincing evidence to favor this scenario [reviewed in (*4*)]. Or perhaps large-scale magnetic fields were directly generated in the early universe, several 100 million years after the Big Bang, when the universe was reionized (*5*) or protogalaxies were formed (*6*, *7*). However, the models still remain uncertain because of the lack of observations of a high-redshift universe.

On the other hand, cosmological mechanisms based on inflation (*8*, *9*) have no difficulty in accounting for the length of coherence; the accelerating expansion of the universe stretches small-scale quantum fluctuations to scales that can exceed the causal horizon. However, because standard electromagnetic fields are conformally coupled to gravity, magnetic fields simply dilute away as the universe expands. Eventually, the amplitudes of the magnetic fields become negligibly small at the end of inflation. To produce substantial primordial magnetic fields during inflation, new coupling—such as exotic coupling of electromagnetic fields to nonstandard particles (*10*–*12*) or gravity (*13*)—must be introduced. This new coupling must amplify magnetic fields against cosmological expansion. Therefore, the nature of generated magnetic fields, such as their amplitude or spectrum, depends strongly on the assumptions built into standard cosmology or particle physics. Moreover, it is argued that almost all the models that generate magnetic fields at the inflationary epoch are ruled out, because they would produce a large amount of gravitational waves before the Big Bang nucleosynthesis, which then makes cosmic expansion faster to bring an overproduction of helium nuclei in the universe (*14*).

In addition to the astrophysical and cosmological mechanisms, there is a third category for the generation of large-scale magnetic fields. Small density fluctuations during the cosmological recombination of hydrogen atoms inevitably induce magnetic fields. Compton and Coulomb scatterings are so efficient that photons, protons, and electrons are approximated to a tightly coupled fluid. If these three kinds of fluids moved in exactly the same way, magnetic fields could not be generated. However, because photons scatter off electrons preferentially, compared with protons, small differences in velocity between protons and electrons are generated, which yields an electric current (*15*, *16*). Moreover, we show that the anisotropic pressure of photons pushes the electrons in a different way from the protons (eq. S2). The rotation of the electric current thus generates magnetic fields. However, the rotation (or vector) mode of perturbations in the linear order is known to be damped away in the expanding universe. Therefore, it is essential to consider the second-order couplings in the Compton scattering term (*17*–*19*). The magnetic fields generated through this process are correlated with temperature fluctuations at the recombination epoch, because the electric current is associated with the density perturbations of photons (Fig. 1).

There are three main contributions to the generation of magnetic fields (eq. S2): (i) the baryon-photon slip term, (ii) the vorticity difference term, and (iii) the anisotropic pressure term. These terms are derived from the fact that electrons are pushed by photons through Compton scattering when velocity differences exist between them or when there is anisotropic pressure from photons. We derive here the power spectrum of magnetic fields (eq. S19) and then perform a numerical calculation to evaluate it. The power spectrum of magnetic fields, $S(k)$, is defined by the expected variance of the Fourier component of magnetic fields

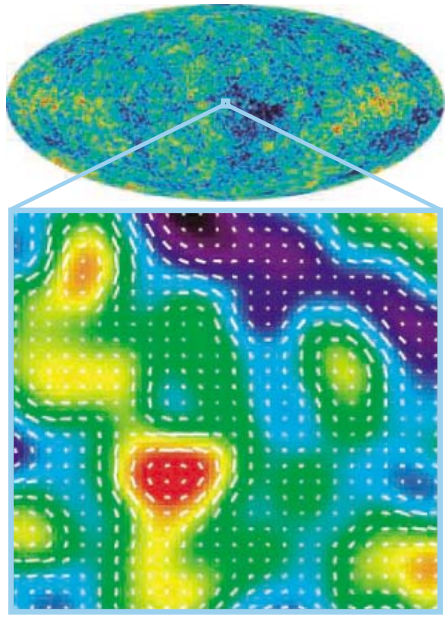

Fig. 1. All-sky map (**top**) of cosmological microwave background anisotropy obtained by the Wilkinson Microwave Anisotropy Probe (WMAP) satellet (*20*) and schematic picture (**bottom**) of cosmological magnetic fields generated from density fluctuations (0.5° × 0.5° sky field, which corresponds to 130 Mpc × 130 Mpc comoving scale). Red regions are hot spots and blue regions are cold spots, with a range of temperatures ~2.725 K ± 200 μK. The magnetic field vectors are shown together with the map. Strong magnetic fields are generated by currents where the gradient of density perturbation in photons is large.

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$\vec{B}(\vec{k})$ as $S(k) \equiv \langle|\vec{B}(\vec{k})|^2\rangle$, where $\vec{k}$ is the wave vector. The component of the field with characteristic wavelength scale λ can then be derived through $B_\lambda \approx \sqrt{[k^3 S(k)/(2\pi^2)]}$, with $\lambda = 2\pi/k$. We consider a standard cosmological model (*20*), which consists of photons, baryons, cold dark matter, neutrinos, and the cosmological constant, and we fix all the cosmological parameters to the standard values (eq. S26). The density perturbations of these parameters were solved numerically for a range of scales from 10 kpc up to 10 Gpc, and they were then integrated to obtain $S(k)$ (eq. S19). We found that the field strength of generated magnetic fields at the time of cosmological recombination can be as large as $10^{-18.1}$ G at 1 Mpc (comoving scale), and it becomes even larger at smaller scales ($10^{-14.1}$ G at 10 kpc) (Fig. 2). After cosmological recombination, no magnetic fields would be generated, because most of the electrons were combined into hydrogen atoms and Compton scattering was no longer efficient. This means that the fields presently have an amplitude of $10^{-24.1}$ G at 1 Mpc ($10^{-20.1}$ G at 10 kpc), because magnetic fields decay adiabatically as the universe expands after their generation. The field strength is large enough to seed the galactic magnetic fields required by the dynamo mechanism, which is typically of the order of 10^{-20} to 10^{-30} G at around the 10-kpc scale (*4*, *21*).

Over the range of scales calculated, the generated magnetic fields increase monotonically with decreasing scale. We found that the field has a spectrum $S(k) \propto k^4$ at scales larger than $\sim 10^{2.5}$ Mpc, which corresponds to super-horizon scales at recombination; $S(k) \propto k^0$ at intermediate scales ($10^{2.5}$ Mpc $< \lambda < 10^{1.5}$ Mpc); and $S(k) \propto k^1$ at scales smaller than ~10 Mpc, where the contribution from the anisotropic stress of photons dominates. This means that the field strength B is proportional to k^2 at scales smaller than 1 Mpc. If the primordial power spectrum of density fluctuations is given by a simple power law, as predicted by inflation (*22*), our result implies that magnetic fields with strength $B \approx 10^{-12.8}$ G arise on a 100-pc comoving scale at $z \approx 10$ (where z is the cosmological redshift). This value helps us to understand the evolution of structures in the high-redshift universe, because those magnetic fields would be strong enough to trigger a magneto-rotational instability in the accretion disks surrounding very first stars (population III stars), and it affect the transport of their angular momentum (*23*). The transport of angular momentum plays an important role for the accretion of matter onto protostars. A typical mass scale of population III stars is key (*23*) for the early reionization and chemical evolution of the universe; therefore, cosmologically generated magnetic fields should be one of the essential ingredients in the model of structure formation in the high-redshift universe.

The behavior of the power spectrum $S(k)$ can be understood by considering the spectra of source terms (eq. S3) at each redshift, from the deep radiation–dominated era to cosmological recombination (Fig. 3). We found that at recombination, both baryon-photon slip and anisotropic stress contribute almost at the same order of magnitude around horizon scales. The contributions from the earlier epochs are dominated by anisotropic stress of photons, and they give rise to larger Fourier components of magnetic fields at smaller scales. Because velocity differences between electrons and photons are suppressed when energy density of radiation dominated in the early universe, anisotropic stress of photons could not be negligible at small scales. We also found that magnetic fields are mainly generated when the baryon-photon fluids undergo acoustic oscillation after crossing the horizon [Fig. 3, left panel, (2)] until photon diffusion processes (*24*) erase the perturbations [Fig. 3, left, (3) and (4)]. The generated spectrum of magnetic fields was obtained by the nonlinear convolution and time integration of these spectra of source terms (eq. S19) but approximately given by the superposition of them at each redshift.

Because the creation of magnetic fields mainly occurs when the modes of density perturbations with the corresponding scale enter the cosmic horizon and become causally connected (Fig. 3), the magnetic fields should exist at small scales below ~10 Mpc, even where the Silk damping effect by diffusion of photons has swept away the density perturbations at the last scattering epoch. Thus, in principle, the detection of magnetic fields

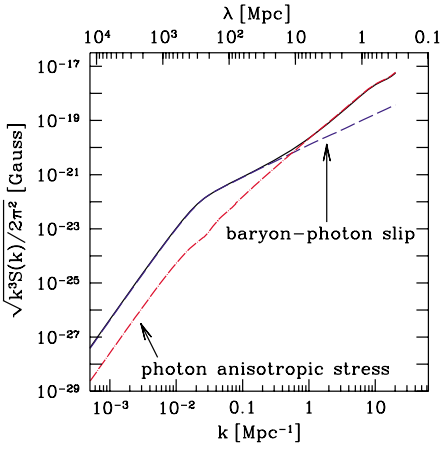

Fig. 2. Spectrum of magnetic fields $S(k)$ generated from cosmological perturbations at cosmological recombination. We plotted $\sqrt{[k^3 S(k)]}$ instead of $S(k)$ to measure in units of gauss. Blue dashed and red dot-dashed lines show contributions from the baryon-photon slip and photon's anisotropic stress (the first and third terms in eq. S2), respectively. The spectrum decays as k^4 at scales larger than that of the cosmic horizon at cosmological recombination. At small scales, the contribution from the anisotropic stress of photons dominates and the spectrum has a slope proportional to k.

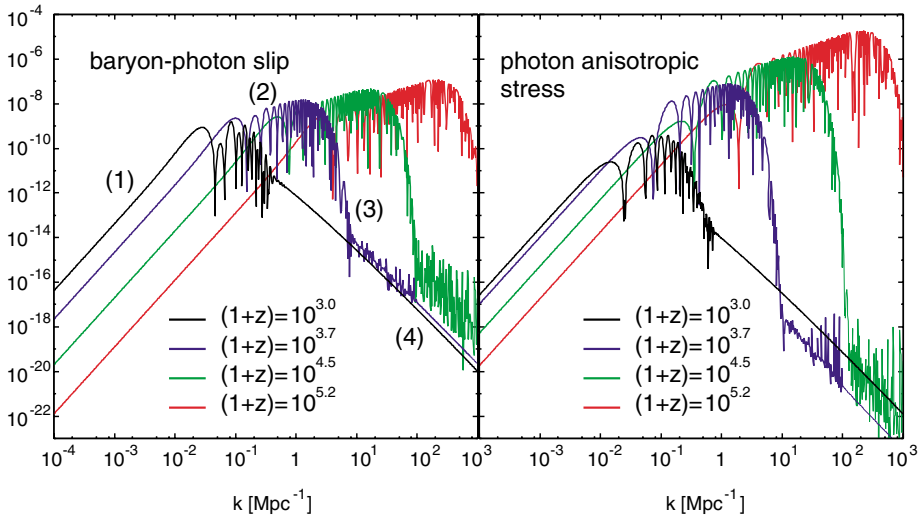

Fig. 3. Plots of source terms in Fourier space per dlog(1 + z), $k^3(\rho_\gamma/H)\delta_\gamma(v_e - v_\gamma)$ (baryon-photon slip contribution; left), and $k^3(\rho_\gamma/H)\Pi_\gamma v_e$ (anisotropic stress contribution; right), at different redshifts. Here, ρ_γ is the energy density of photons, H is the Hubble parameter, δ_γ is the energy density fluctuation of photons, v_e is the bulk velocity of electrons, v_γ is the bulk velocity of photons, and Π_γ is the anisotropic stress of photons. The magnetic field spectrum (Fig. 2) is obtained by time and k-space convolution integrals of these spectra (eq. S19). The spectrum at each redshift is divided into four parts from large scales to small ones (blue line in the left panel): (1) featureless primordial power law spectrum at super horizon scales, (2) acoustic oscillation spectrum at subhorizon scales, (3) damping spectrum at diffusion scales, and (4) power law phase after the diffusion damping before recombination (*29*). These spectra indicate that magnetic fields are created when the modes of perturbations come across the cosmic horizon and undergo acoustic oscillations. The redshifts in the figure are chosen only for illustration.

below the ~10-Mpc scales calculated here would tell us about density perturbations in photons (and baryons) in the early universe, even at scales smaller than the diffusion scale at recombination. In this sense, the magnetic field generated by this mechanism can be regarded as a fossil of density perturbations in the early universe, whose signature in photons and baryons has been lost. Therefore, this result provides the possibility of probing observations on how density perturbations in photons have evolved and been swept away at these small scales, where no one can, in principle, probe directly through photons.

The amplitude of the cosmologically generated magnetic fields is too small to be observed directly through polarization effects or synchrotron emission. However, magnetic fields with such small amplitude may be detected by gamma ray burst observations (*25*) through the delay of the arrival time of gamma ray photons due to the magnetic deflection of high-energy electrons responsible for such gamma ray photons (*26*, *27*). We suggest that, because the weak magnetic fields should inevitably be generated from cosmological perturbations as presented here, and because they should exist all over the universe even in the intercluster fields, then the weak fields should be detectable by future high-energy gamma ray experiments, such as GLAST (the Gamma Ray Large Area Space Telescope) (*28*).

Although the power of B increases as k^2 on small scales (Fig. 2), the diffusion due to Coulomb scattering between electrons and protons damps the magnetic fields around $k \sim 10^{12}$ Mpc^{-1} $[(1 + z)/(10^4)]^{7/4}$. Therefore, the energy density in magnetic fields remains finite. Because magnetic fields at smaller scales result from density perturbations in the early universe, one needs to take into consideration the high-energy effects neglected in the collision term: e.g., relativistic corrections for the energy of electrons and weak interactions along with the Compton scattering. These effects would become important when the temperature of the universe was above ~1 MeV, which corresponds to the comoving wave number ~10^5 Mpc^{-1}. These effects can be safely neglected at the scales considered here.

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Reductive Cyclotrimerization of Carbon Monoxide to the Deltate Dianion by an Organometallic Uranium Complex

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Despite the long history of the Fischer-Tropsch reaction, carbon monoxide has proven remarkably resistant to selective homologation under mild conditions. Here, we find that an organouranium(III) complex induces efficient reductive trimerization of carbon monoxide at room temperature and pressure. The result is a triangular, cyclic $C_3O_3^{2-}$, or deltate, dianion held between two uranium(IV) units. The bonding within the $C_3O_3^{2-}$ unit and its coordination to the two U centers have been analyzed by x-ray diffraction and density functional theory computational studies, which show a stabilizing C-C agostic interaction between the C_3 core and one U center. Solution nuclear magnetic resonance studies reveal a rapid equilibration of the deltate unit between the U centers.

Carbon monoxide, usually obtained from coal or natural gas, is an important industrial feedstock for the production of hydrocarbons and oxygenates, especially in times of limited crude oil supply, through the Fischer-Tropsch process. This process uses CO/H_2 as the feedstock and is catalyzed by both homogeneous and heterogeneous systems (*1*). Metal-catalyzed coupling of CO with unsaturated organic substrates (e.g., hydroformylation catalysis) represents a further industrially important method of C-C bond formation (*2*). The concept of reductively homologating CO directly to form larger units of $(CO)_n$ is an appealing one; however, the strength of the CO triple bond has made this route difficult to achieve effectively. The cyclic, aromatic, oxocarbon anions $C_nO_n^{2-}$ (n = 3 to 6 in Scheme 1) have fascinated organic chemists for many years (*3*, *4*) and, in principle, are accessible by such a route. This would provide a facile synthesis of fused carbon ring systems as building blocks for further elaboration to more complex organic molecules (e.g., possible ring expansion of **A** for prostaglandin synthesis).

The formation of salts of the croconate and rhodizonate dianions—**C** and **D**—from the reduction of CO by molten alkali metals [which also affords salts of the linear $C_2O_2^{2-}$ ethynediolate dianion (*5*)] was first reported in the early part of the 19th century (*6*), and there is some nuclear magnetic resonance (NMR) evidence for formation of trace amounts of the deltate dianion **A** in a complex mixture of products from the reaction of CO with Na-K alloy in tetrahydrofuran (THF) (*7*). However, salts of the trimer **A** have eluded crystallographic characterization or selective metal-mediated synthesis. The squarate dianion **B** has been generated by electrochemical methods using very high pressures of CO (*8*), and surface catalysis routes using alkaline earth oxides yield mixtures of various $(CO)_n^{2-}$ (n = 2 to 6) species (*9*).

The idea of using low-valent f-element complexes, which combine high reduction po-

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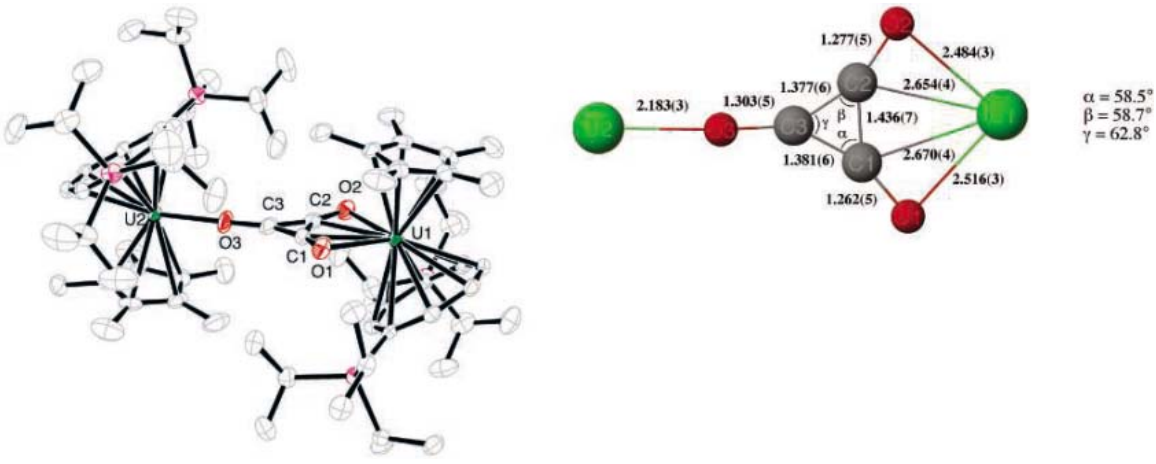
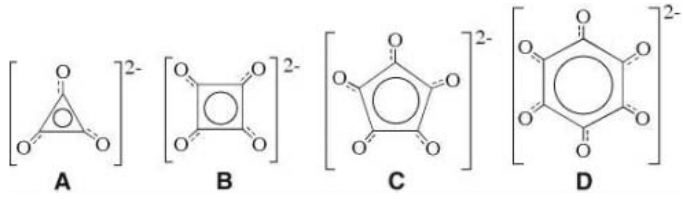


Fig. 1. (**Left**) The molecular structure of **2** (thermal ellipsoids at 50%). (**Right**) View of core structure (distances in Å; values in parentheses are errors in the last significant digit).

tentials with solubilizing ligands, to give isolable $(CO)_n^{2-}$ species was demonstrated by Evans *et al.*, who reported that $[Sm(\eta\text{-}Cp^*)_2(THF)_2]$ (Cp* is pentamethylcyclopentadienyl) will react under a 90 pounds per square inch overpressure of CO to give a doubly charged ketene carboxylate unit $(O_2CCCO)^{2-}$ bound to samarium (*10*). Here, we extend that idea to selectively synthesize and structurally characterize the deltate dianion **A** directly from CO under very mild conditions. Specifically, reductive cyclotrimerization of CO is induced by an organometallic U(III) complex.

There is considerable current interest in the binding of small molecules to U(III) centers, e.g., CO (*11*), CO_2 (*12*), and N_2 (*13*). As part of our continuing investigation into reduction chemistry by low-valent uranium complexes, we recently reported the reversible binding and concomitant double reduction of dinitrogen by the pentalene complex $[U(\eta\text{-}C_8H_4^{\dagger})(\eta\text{-}Cp^*)]$ (*14*) [† is 1,4-bis(tri-isopropylsilyl)]. We explored the reactivity of the related silylated cyclooctatetraene (COT) complex with CO; the use of bulky tri-isopropylsilyl substituents on the COT ring provides steric protection for reactive metal centers and imparts high crystallinity to the derived metal complexes. The THF adduct $[U(\eta\text{-}COT^{\dagger})(\eta\text{-}Cp^*)(THF)]$ **1** was obtained by a route similar to that employed for the unsilylated analog $[U(\eta\text{-}COT)(\eta\text{-}Cp^*)(THF)]$ (*15*); reaction of UI_3 with KCp* in THF yielded $[UI_2Cp^*(THF)_3]$, which was treated in situ with 0.8 equivalent of $K_2[COT^{\dagger}]$ in THF to give **1** as a dark purple, crystalline material in 39% overall yield after recrystallization from pentane at –50°C (Scheme 2). Compound **1** has been structurally characterized by x-ray diffraction (*16*).

Compound **1** reacts with ambient pressures (1 bar) of CO in pentane to give the dimeric U(IV) complex **2** in 40% isolated yield. Crystallization from diethyl ether at –50°C gave dark red needles of **2** suitable for x-ray diffraction, which revealed a reductively homologated CO trimer held between the two uranium(IV) centers (Scheme 2 and Fig. 1) (*17*).



Scheme 1.

The structure shows a cyclic (C_3O_3) moiety bound through the oxygen atoms in a bridging $\eta^1{:}\eta^2$ fashion to the two uranium centers. Metal-ligand bond lengths within the $[U(\eta\text{-}COT^{\dagger})(\eta\text{-}Cp^*)]$ fragments reflect the steric hindrance around the respective uranium centers, i.e., average U-$COT^{\dagger}$ and U-Cp* ring carbon distances are slightly longer around the η^2-bound U1 center (2.696 Å and 2.775 Å, respectively) than the η^1-bound U2 (2.656 Å and 2.740 Å). We propose an oxidation state of (IV) for both U centers in **2** on the basis of the density functional theory (DFT) computational studies below. Oxidation from U(III) in **1** to U(IV) in **2** is not, however, reflected in the structural parameters; such changes were similarly lacking in the conversion of the pentalene complex $[U(\eta\text{-}C_8H_4^{\dagger})(\eta\text{-}Cp^*)]$ to $[U(\eta\text{-}C_8H_4^{\dagger})(\eta\text{-}Cp^*)]_2(\mu\text{-}\eta^2{:}\eta^2\text{-}N_2)$ (*14*), presumably due to steric congestion in the uranium(IV) dimers.

Because the other bond distances and angles associated with the two $[U(\eta\text{-}COT^{\dagger})(\eta\text{-}Cp^*)]$ fragments are unexceptional, we now focus on the $U(C_3O_3)U$ core. The (C_3O_3) unit is planar, with the two uranium centers lying slightly above (U2, 0.0906 Å) and below (U1, 0.1747 Å) this plane. The η^1-uranium-oxygen distance (U2-O3) of 2.183(3) Å is slightly longer than those found in U(IV) aryloxides {e.g., 2.120 Å in $[U(\eta\text{-}Cp^*)_3(OPh)]$} (Ph, is phenyl) (*18*), but U1-O1 [2.516(3) Å] and U1-O2 [2.484(3) Å] are significantly longer. The C-O bond lengths [which lie between typical values for single C-O (1.43 Å) and double C=O (1.21 Å) bonds] show complementary variations to the U-O bond lengths: C3-O3 is somewhat longer than C1-O1 and C2-O2 (Fig. 1).

The triangular C_3 skeleton in **2** is also noticeably distorted (Fig. 1), with two short C-C bonds and one long one, the latter showing some interaction with U1. We probed the bonding in this deltate diuranium complex using DFT. The unsubstituted compound $[U_2(\eta\text{-}COT)_2(\eta\text{-}Cp)_2(\mu\text{-}\eta^1{:}\eta^2\text{-}C_3O_3)]$ **II** was used as a model for compound **2**. The experimental coordinates determined from the solid state structure of **2** were used to describe the initial geometry of **II**, and a geometry optimization was performed with no symmetry constraints. In addition, a fragment analysis was conducted in which **II** was divided into two U(η-COT)(η-Cp) fragments and a C_3O_3 fragment. It is often difficult to achieve self-consistent field (SCF) convergence in calculations involving open-shell actinides, because there are a large number of orbitals close to the Fermi surface of the molecule. In this case, the geometry optimization was carried out as a spin-restricted calculation, with electron density smeared over the frontier orbitals to assist SCF convergence (*16*). As a result, the frontier orbitals have fractional occupancy; however, the molecular geometry is typically not very sensitive to the exact description of the f electrons (*19*).

There was good agreement between the experimental and calculated bond distances and angles in the $U(C_3O_3)U$ core of **II** (table S1), demonstrating that DFT can accurately predict the structure of **2**. The calculated structure reproduced the distortions in the C_3 core, with two short C-C bonds (C2-C3, 1.41 Å; C1-C3, 1.41 Å) and one long bond (C1-C2, 1.47 Å). The C_3O_3 unit was planar, and the C-O and U-O bond distances followed the pattern observed in the solid-state structure.

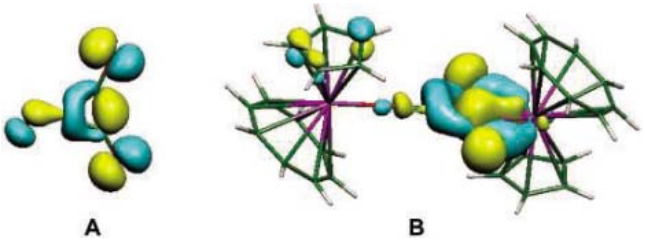
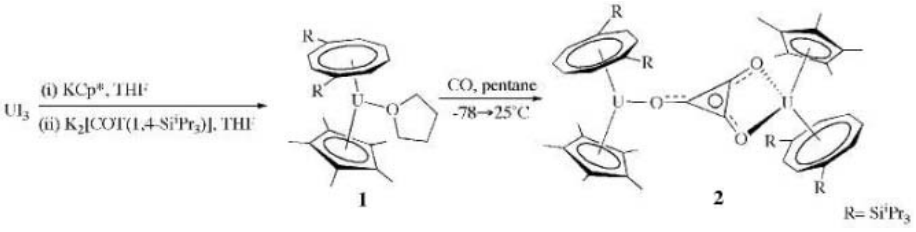


Fig. 2. (**A**) Key orbital of the distorted deltate dianion involved in an agostic interaction with the U center. Its parentage is one of the degenerate e′ C-C bonding orbitals of $(C_3O_3)^{2-}$. (**B**) MO showing the agostic interaction between the deltate dianion and an f orbital on U(I). Details of these orbitals are given in table S2.



Scheme 2.

There were minor differences between the calculated and experimental U-COT and U-Cp bond lengths and angles, suggesting that these interactions are controlled by steric interactions; however, the discrepancy is not sufficiently great to invalidate the model. The calculations indicate that each U is best described as having two electrons localized in 5f orbitals. Thus, the U configuration is consistent with U(IV); chemical plausibility and theoretical studies (*20*) also suggest that the deltate structure is not likely to be stable in a neutral state, whereas it is known in a dianionic 2-state. The COT and Cp ligands bind to the U centers as expected, with primarily a π interaction between U and Cp and a δ interaction between U and COT.

Previous work has shown that the highest occupied molecular orbitals (MOs) of the relaxed $(C_3O_3)^{2-}$ anion (in D_{3h} symmetry) are a π orbital of a_2'' symmetry (which confers aromaticity on the dianion) and a degenerate pair of C-C bonding orbitals (e′), which have C-O antibonding character (*21*). On binding to the two U fragments, the symmetry of the $(C_3O_3)^{2-}$ anion is lowered, but the MOs of the distorted fragment are clearly related to the MOs of the relaxed parent fragment in D_{3h} symmetry. Fragment analysis indicates that four MOs of the deltate anion have a notable interaction with the two U-containing fragments (table S2 and fig. S1). The primary interaction involves donation of electron density from the oxygen atoms of the $(C_3O_3)^{2-}$ ligand to the U atoms. However, a more complex interaction occurs between the lengthened C-C bond and a U f orbital. Figure 2 shows a representation of this parent $(C_3O_3)^{2-}$ MO, which shows that the orbital primarily consists of the lone pairs on O1 and O2 and a bonding interaction between C1 and C2. The parent orbital is bonding between C1 and C2 and is antibonding with respect to the lone pairs on both O1 and O2. This orbital interacts with an f orbital on U1 to form an MO of **II** (which is also shown in Fig. 2). This MO comprises not only a bonding interaction between U1 and O1, O2 but also (and of more importance) a bonding interaction between U1 and C1, C2. The net effect of this second interaction is to weaken the C1-C2 bond, because some of the electron density between C1 and C2 is shared with U1, and to strengthen the C1-O1 and C2-O2 bonds, because the antibonding electron density is decreased. Thus, a C-C agostic interaction appears to be responsible for the distortion in the C_3 core observed in **2**. The highly nodal characteristics of the f orbital on U enable such an interaction and, thus, actinides may be particularly suited to stabilize such CO homologs. This finding suggests that tuning the ligand environment around U(III) may give access to higher homologs of the deltate dianion.

^{1}H NMR solution studies of **2** in d_8-toluene do not reflect the asymmetry present in the solid-state structure (Fig. 1). Only single environments for the Cp* ligands and the $C_8H_6^{\dagger}$ ligands are observed at 25°C, indicating a rapid (milliseconds on the NMR time scale) fluxional process leading to equilibration of the deltate unit between U1 and U2 at this temperature. This finding was confirmed by a study of the isotopically labeled complex $[U(\eta\text{-}COT^{\dagger})(\eta\text{-}Cp^*)]_2(\mu\text{-}\eta^1\text{:}\eta^2\text{-}^{13}C_3O_3)$ **2-^{13}CO**, prepared by reaction of **1** with ^{13}CO: **2-^{13}CO** displays a singlet ($\nu_{1/2}$ 12 Hz) at δ 225 parts per million for the deltate carbons in the ^{13}C NMR spectrum at 25°C. At −100°C, both ^{1}H and ^{13}C spectra for **2-^{13}CO** display the expected changes in chemical shifts with temperature for a paramagnet, but, apart from considerable broadening of the signals, there is no evidence for freezing out of the fluxional process at this temperature. Hence, these data do not allow us to distinguish between the two likely mechanisms for the equilibration of the C_3O_3 unit in **2**, i.e., a "windshield wiper" motion of O1 (or O2) between U1 and U2, versus a rotational "propeller" mechanism involving all three oxygen atoms.

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17. Data collection was performed at 173(2) K on an Enraf-Nonius Kappa charge-coupled device diffractometer with graphite-monochromated MoKα radiation (λ = 0.71073 Å). The molecular structure was solved by direct methods and refined on F^2 by full-matrix least squares techniques. For **2.Et2O**: triclinic, $P\bar{1}$ (No. 2), a = 10.7193(2) Å, b = 16.6281(2) Å, c = 23.2496(4) Å, α = 99.768(1)°, β = 95.816(1)°, γ = 91.500(1)°, V = 4058.97(11) Å^3, Z = 2, $R1$ = 0.034, $wR2$ = 0.070.
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22. We thank the Engineering and Physical Sciences Research Council for financial support of this work. Metrical data for **1** and **2** are available from the Cambridge Crystallographic Data Centre under reference numbers CCDC-292279 and CCDC-292280, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/829/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3
References

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A Molecular Jump Mechanism of Water Reorientation

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Despite long study, a molecular picture of the mechanism of water reorientation is still lacking. Using numerical simulations, we find support for a pathway in which the rotating water molecule breaks a hydrogen bond (H-bond) with an overcoordinated first-shell neighbor to form an H-bond with an undercoordinated second-shell neighbor. The H-bond cleavage and the molecular reorientation occur concertedly and not successively as usually considered. This water reorientation mechanism involves large-amplitude angular jumps, rather than the commonly accepted sequence of small diffusive steps, and therefore calls for reinterpretation of many experimental data wherein water rotational relaxation is assumed to be diffusive.

Most of the remarkable properties of liquid water stem from its ability to form dynamic, labile H-bond networks (*1*–*5*) whose connectivity changes constantly, especially because of the rotation of individual water molecules. Water reorientation is of importance in a wide range of processes, including proton transfer (*6*, *7*) and proton transport (*8*, *9*), where it can be the rate-limiting step, and hydration of macromolecules such as proteins, where the water dynamics are essential for protein function (*2*). This reorientation has long attracted experimental interest (*10*), with much very recent activity (*11*–*17*) prompted by progress in time-resolved infrared spectroscopy that now allows detailed study of H-bond dynamics in liquid water. The attained femtosecond time resolution is well adapted to the rapid local structural changes in the H-bond network. Several of these studies (*11*–*15*) have focused on the reorientation kinetics of liquid water or its isotopic variants, extending previous studies based on dielectric relaxation, terahertz (THz) spectroscopy (*17*), optical and Raman-induced Kerr-effect spectroscopy (*16*), nuclear magnetic resonance (NMR) (*16*), and neutron scattering (*10*).

The observed picosecond orientational relaxation of water molecules is usually believed to be related to H-bond breaking and forming, but the specific sequence of events remains unclear. Several mechanisms have been suggested (*1*), involving, e.g., "flickering clusters," which have been argued not to be applicable (*1*), or icelike orientational defects, or especially the Debye small-step diffusion model, but a molecular mechanism consistent with all experimental data is elusive. Although the diffusion model has been most often employed in discussions of water reorientation, several authors (*1*, *2*, *16*, *18*) have expressed doubts about its validity.

It is commonly assumed (*1*, *2*) that to reorient markedly, a water molecule must break at least the H-bond involving the rotating hydrogen. We therefore first focus on the molecular mechanism describing the migration of this H-bond donor site from one accepting water molecule to another. We then show that this exchange phenomenon dominates the observed reorientation dynamics.

Classical molecular dynamics (MD) simulations provide an incisive tool for this study, because they provide a molecular picture of the mechanism while giving results that can be compared with experimental measurements, e.g., from time-resolved infrared spectroscopy. The simulations are performed with 256 H_2O molecules in a periodic cubic box with Ewald summation for long-range forces, employing the rigid simple point charge extended (SPC-E) model to describe the water molecules, with a 0.5-fs timestep. This computational scheme and model have been shown to yield good agreement with experimental results (*12*, *14*, *18*).

To detect H-bond breaking and forming events along a dynamical trajectory, criteria characterizing the existence of an H-bond must first be chosen. We have adopted the widely used geometric definition (*5*, *12*, *19*, *20*): $R_{OO} < 3.5$ Å, $\theta_{HOO} < 30°$, where R_{OO} is the distance between the donor and acceptor oxygen atoms, and θ_{HOO} is the angle between the OH bond and the OO vectors.

After a 50-ps equilibration period at 300 K and a density of 1 g/cm^3, a 75-ps classical MD trajectory for pure H_2O is generated in the microcanonical ensemble. We record all the times when a hydrogen atom changes the acceptor oxygen with which it forms an H-bond and collect more than 16,000 such H-bond "switches"; these switches are a feature common to all water molecules, i.e., they do not represent a subensemble. The switching comprises three steps: (i) the breaking of an initial H-bond formed between two water molecules $HO^*\text{-}H^*\cdots O^aH_2$; (ii) the rotation of the hydrogen H* on the central water molecule; and (iii) the formation of a new H-bond with a different water molecule $HO^*\text{-}H^*\cdots O^bH_2$. We select all the sequences in the trajectory occurring before and after a switching event, provided that H* is H-bonded to either O^a for $t < 0$ or to O^b for $t > 0$ (because near $t \approx 0$, the $O^aO^*H^*$ and $O^bO^*H^*$ angles are close to 30°, the H-bond criteria for the definition of this ensemble are extended to $R_{OO} < 4.0$ Å, $\theta_{HOO} < 50°$). Then we calculate the average over the different trajectories of the time evolution for different key quantities (Fig. 1).

We monitor the oxygen-oxygen distances R_{O^*Oa} and R_{O^*Ob}, together with the angle θ between the projection of the O*H* vector on the $O^*O^aO^b$ plane and the $O^aO^*O^b$ angle bisector (Fig. 2). This definition of θ renders it independent of both any motion of H* out of the $O^*O^aO^b$ plane and any $O^aO^*O^b$ bending. When $\theta = 0°$, H* is equidistant from O^a and O^b.

From more than 16,000 averaged successful trajectories, the flipping of H* from O^a toward

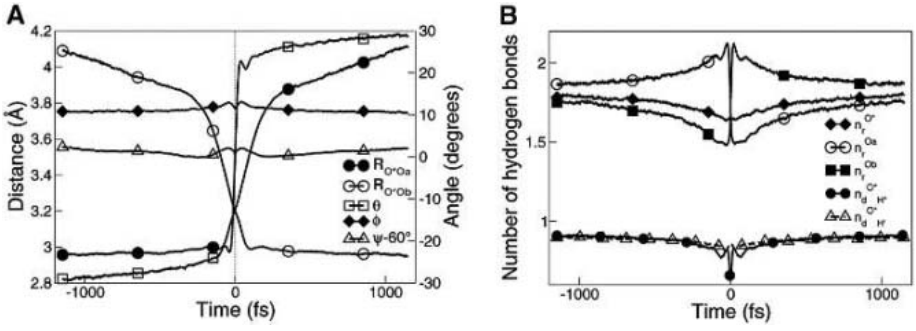

Fig. 1. Time evolution, centered on the H-bond switching event, of (**A**) different geometric quantities and (**B**) the number of H-bonds. (A) R_{O^*Oa}, R_{O^*Ob} distances and θ angle defined in text and Fig. 2; φ is the out-of-plane angle between the O*H* bond and the $O^*O^aO^b$ plane; ψ is the $O^aO^*O^b$ angle, shifted by 60°. θ oscillations between $t \approx -100$ fs and $t \approx +100$ fs arise from the coherent averaging of the trajectories centered on $t = 0$ when $\theta = 0$. The period is ~140 fs, about twice that associated with the water 440 cm^{-1} librational band (*10*), consistent with an angular range about double the appropriate range in either the reactant or product H-bonding conformation. (B) $n_r^{O^*}$, n_r^{Oa} and n_r^{Ob} are the numbers of H-bonds accepted, respectively, by O*, O^a, and O^b; $n_d^{O^*}{}_{H'}$ and $n_d^{O^*}{}_{H^*}$ are the numbers of H-bonds donated by O* through its H′ and H* hydrogens, respectively. O^a for $t \ll 0$ and O^b for $t \gg 0$ are more coordinated than the average bulk oxygen, because H* is always H-bonded to either O^a or O^b in the configurations contributing to the average.

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O^b occurs in approximately 250 fs, on average from $\theta = -29°$ to $\theta = +29°$. The actual angular jump only takes place once the two oxygen atoms O^a and O^b are equidistant from O*: $R_{O^*O^a} = R_{O^*O^b}$ (Fig. 1). The θ angle is therefore a fast coordinate that adapts rapidly to the environment. The rotation of H* occurs nearly within the $O^*O^aO^b$ plane, because the angle ϕ between the O*H* direction and the $O^*O^aO^b$ plane remains approximately constant and equal to 11°. The other H-bonds involving O* remain intact during the rotation: The H-bond donated by the other hydrogen, labeled H′, attached to O*, is unaffected, and the two H-bonds accepted by O* are only slightly weakened (Fig. 1).

By following the averaged trajectories backward in time from the midpoint $t = 0$, defined by $\theta = 0$, we see why the condition $R_{O^*O^a} = R_{O^*O^b}$ occurs. The trajectories show that, before reaching the situation where $R_{O^*O^a} = R_{O^*O^b}$, O^a is H-bonded to H* and lies in the O* first hydration shell ($R_{O^*O^a} \approx 2.95$ Å), whereas O^b is initially located on the inner side of the O* second hydration shell ($R_{O^*O^b} \approx 4.1$ Å). Both O^a and O^b accept $n^{bulk} \approx 1.8$ H-bonds, as does any water molecule in the bulk on average. The departure of O^a away from O* and the motion of O^b toward O* are initiated by changes in the numbers of H-bonds accepted by O^a and O^b as a result of fluctuations in the bulk H-bond network. The coordination of O^a increases markedly, by close to 0.3 in excess of n^{bulk} H-bonds, and therefore O^a moves away from the H-bond donated by H*. In contrast, the coordination of O^b drops by approximately –0.3 from n^{bulk}, attracting O^b toward H* to regain coordination. This difference in coordination is at the origin of the motion of O^a and O^b, leading to the $R_{O^*O^a} = R_{O^*O^b}$ conformation, where the H* rotation takes place; it is also analogous to the slow environment reorganization coordinate that has been put forward as the reaction coordinate for proton transfers in solution (*7*).

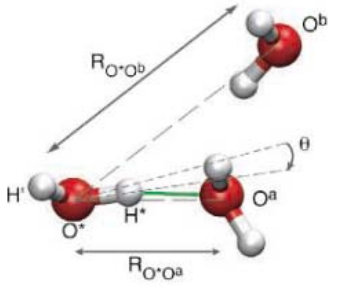

Fig. 2. Scheme of the different geometric coordinates. $R_{O^*O^a}$ and $R_{O^*O^b}$ are the oxygen-oxygen distances between the rotating water and the initial and final H-bond acceptor, respectively. θ is the angle between the projection of the O*H* vector on the $O^*O^aO^b$ plane and the bisector of the $O^aO^*O^b$ angle. All dashed lines lie within the $O^*O^aO^b$ plane.

The different stages of our computed mechanism are represented schematically in Fig. 3. This molecular mechanism indicates that the reorientation of a water molecule occurs by large amplitude rotations, or jumps. The O*-H* direction oscillates around the O*-H*···O^a H-bond axis until collective fluctuations of the H-bond network (*3*) reorganize the environment in such a way that when H* rotates out of the H-bond with O^a, it can immediately form a new H-bond with O^b, thus lowering the energy cost associated with the H-bond breaking. As stressed above, the two coordination number changes are key in this reorganization. The first step in the reorientation is, therefore, the formation of an H-bond around O^a and the cleavage of an H-bond around O^b in the second hydration shell around the rotating water. This result contradicts a standard assumption, namely that the first step is instead the breaking of the H-bond such that a dangling, i.e., non–H-bonded, O*H* is formed, which will subsequently reorient (*1*, *2*). The very recently established unstable character of the dangling H-bond in fact leaves little time for such subsequent reorientation (*20*).

In the present mechanism, an unstable dangling O*H* does not appear. Instead, the key unstable structure is that of the transition state, whose structure exhibits a bifurcated H-bond, with a five-coordinate rotating water (*21*). This last result confirms previous findings that five-coordinate water molecules have higher translational and rotational mobility (*22*), and it provides the molecular mechanism for the formation of these defects. In addition, such a jump mechanism for water rotation is in strong contrast with the diffusive reorientation commonly assumed in the interpretation of time-resolved infrared (*13*–*15*), Raman-induced Kerr-effect (*16*), NMR (*16*) or THz (*17*) spectroscopies, and neutron-scattering experiments (*10*). It also differs from the mechanism determined in isolated water clusters (*23*) because of the constraints imposed by the H-bond network in the bulk. Although the reorientation mechanism suggested in ice (*1*) also involves large angular jumps, it strongly differs from the present mechanism, because the rotating water keeps the same neighbors in its tetrahedral environment and the D/L orientational defects diffuse independently, whereas here the reorientation requires an overcoordinated/undercoordinated defect pair around the rotating water.

Our proposed mechanism can be related to the orientational relaxation times obtained from experiments or MD simulations through the jump model developed by Ivanov (*24*). The water molecule is approximated as spherical so that rotations about different axes occur with the same probability [although water rotation is anisotropic, the OH rotational diffusion constant is close to the averaged isotropic rotational diffusion constant (*25*)]. The molecular orientation is assumed to be "frozen" between changes induced by instantaneous constant-amplitude orientation jumps. Two parameters apply in this model, the first being the average amplitude of the rotational jumps, which we determined to be $\theta_0 = 60°$ from the average $O^aO^*O^b$ angle when the jump occurs (Fig. 1). Because the O*-H* bond oscillates in the direction of O^a for $t < 0$ and in the direction of O^b for $t > 0$, the $O^aO^*O^b$ angle at $t = 0$ gives the angular jump amplitude

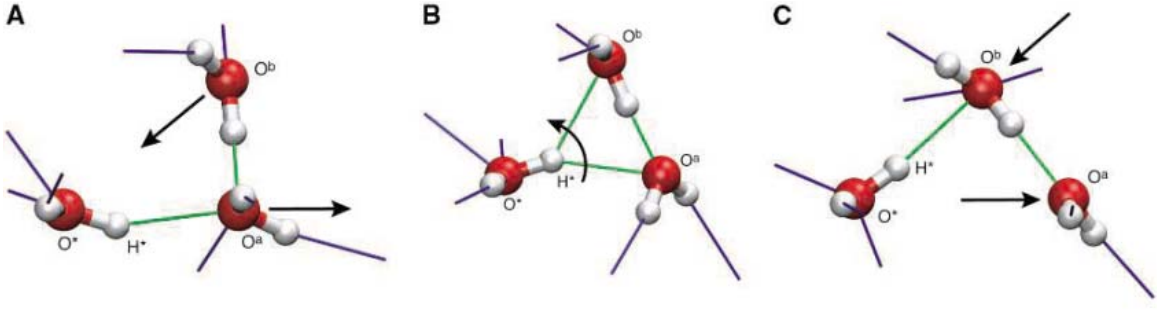

Fig. 3. Scheme of the different steps in the computed H-bond exchange mechanism. Each frame is a snapshot of an actual H-bond switching event in the simulations. The green sticks designate the H-bonds within the O*, O^a, O^b water trimer, and the blue sticks represent the H-bonds between the trimer and the surrounding water molecules, which are not shown. The black arrows show the key motions. (**A**) H* is H-bonded to O^a in its first hydration shell, with O^b farther away in the second hydration shell; because of collective fluctuations in the H-bond network, O^a is overcoordinated, whereas O^b is undercoordinated. This conformation leads to the O^a motion away from O* and O^b motion toward O*. The configuration displayed here with O^a and O^b H-bonded occurs in ~60% of the observed switches. (**B**) O^a and O^b are equidistant from O*, and H* flips from O^a toward O^b; O^a and O^b have the same coordination number. (**C**) O^b forms an H-bond with H* and becomes overcoordinated, whereas O^a has lost an H-bond and is now undercoordinated; the trimer is in a situation similar to (A), with O^a and O^b interchanged; fluctuations of the H-bond network will allow the configuration to relax to equilibrium.

$\theta_0 = 60°$; Fig. 1 shows that the average $O^aO^*O^b$ angle varies little during the switch.

The second parameter is the frequency $1/\tau_0$ of these jumps, which corresponds to the rate constant of the H-bond switching event that we have monitored. This rate constant is extracted from our simulations by calculating the correlation function $[1 - \langle n_a(0) n_b(t)\rangle]$, where n_a is 1 when H* is H-bonded to O^a and 0 otherwise, and n_b is 1 when H* is H-bonded to O^b and 0 otherwise. In addition, absorbing conditions are used for n_b so that when a new H-bond has formed, it remains intact. We thus discard any contribution from the back reaction or from the subsequent rupture of the O^b H-bond, so that the extracted time is associated with the forward rate constant for the H-bond switching (*19*). This correlation function exhibits a mono-exponential decay with a characteristic time $\tau_0 = 1.8$ ps (Fig. 4A). The Ivanov jump model then provides the τ_n^{jump} characteristic times associated with the different rotational correlation functions $\langle P_n[\mathbf{u}_{OH}(0)\cdot\mathbf{u}_{OH}(t)]\rangle$ where P_n is the nth order Legendre polynomial and $\mathbf{u}_{OH}$ the direction of the rotating OH bond

$$\tau_n^{jump} = \tau_0 \Big/ \left(1 - \frac{1}{2n+1}\,\frac{\sin(n+1/2)\theta_0}{\sin\theta_0/2}\right) \quad (1)$$

If the angular jump is very small, this relation reduces to the diffusive reorientation case $\tau_n^{dif} = 1/[n(n+1)D_{OH}]$, with the OH rotational diffusion coefficient $D_{OH} = \theta_0^2/6\tau_0$.

As noted above, the Ivanov jump model assumes that the O*-H* orientation is "frozen" between jumps. Figure 4B displays the orientation correlation functions calculated over all the trajectories during the time intervals between two successive H-bond switches and indicates that there is instead a relatively slow reorientation process occurring between jumps (compare with Fig. 4A). Figure 4B shows that this slow reorientation can be understood in terms of the coupling of the OH* to the OO axis in the initially H-bonded pair, which will be shown below to be diffusive in character. To incorporate this slower process in our description within an extended jump model, we can employ the relation (*26*) for orientation correlations involving several statistically independent reorientation sources

$$\langle P_n(\mathbf{u}_{OH}(0)\cdot\mathbf{u}_{OH}(t))\rangle = \langle P_n(\hat{\mathbf{u}}_{OH}(0)\cdot\hat{\mathbf{u}}_{OH}(t))\rangle \times \langle P_n(\mathbf{u}_{OO}(0)\cdot\mathbf{u}_{OO}(t))\rangle \quad (2)$$

in the present case connecting the orientational correlation functions to those for the OO axis frame and for the "internal" switch. Here $\hat{\mathbf{u}}_{OH}$ is the direction of the rotating OH bond in the OO frame, and $\mathbf{u}_{OO}$ is the direction of the OO axis in the laboratory frame. Because, from Fig. 4, each of the latter functions is exponential in time past a very short transient period (~400 fs), the time dependence of the nth Legendre polynomial is exponential, with a decay time given by

$$\frac{1}{\tau_n} = \frac{1}{\tau_n^{jump}} + \frac{1}{\tau_n^{OO}} \quad (3)$$

where τ_n^{OO} is the exponential decay time associated with the OO frame between jumps determined from the simulation results in Fig. 4B.

Table 1 summarizes the different values obtained for the orientational relaxation times, either from experiments (if available), from our MD simulations, from the diffusive model with the OH rotational diffusion constant calculated from our MD simulations independently of any consideration of the jumps (*25*), from jump model calculations using the θ_0 and τ_0 values determined by the simulations, from the OO frame rotational reorientation between H-bond switches, or from the extended jump model combining jumps and OO reorientation. The MD results are shown to be fully consistent with the available experimental relaxation time values. The calculated τ_2 value is 2.0 ps, in agreement with the 1.7 to 2.6 ps range of values obtained from different experimental techniques (*12*, *13*, *15*, *16*), thus validating our method. Inspection of the τ_n values predicted by the OH diffusive model shows very poor agreement with both the experiments and the MD simulations; the standard diffusive value of 3 for the τ_1/τ_2 ratio agrees only very approximately with the MD ratio of 2.1, which has been in the past considered to be sufficient for a confirmation of the diffusive behavior. Regarding the extended jump model, the explicit inclusion of the OO reorientation between jumps shortens only slightly the predicted reorientation times of the simple jump model, because the reorientation is dominated by the fast jumps with respect to the slow OO reorientation. (The values of the τ_1/τ_2 and τ_1/τ_3 ratios of 3.0 and 5.5 for the latter OO reorientation tend to indicate a diffusive process for it.) For the τ_n values given by the extended jump model, the agreement with the MD results is now excellent, and the predicted τ_1/τ_2 ratio of 2.5 is in better agreement with the MD results than the diffusive ratio.

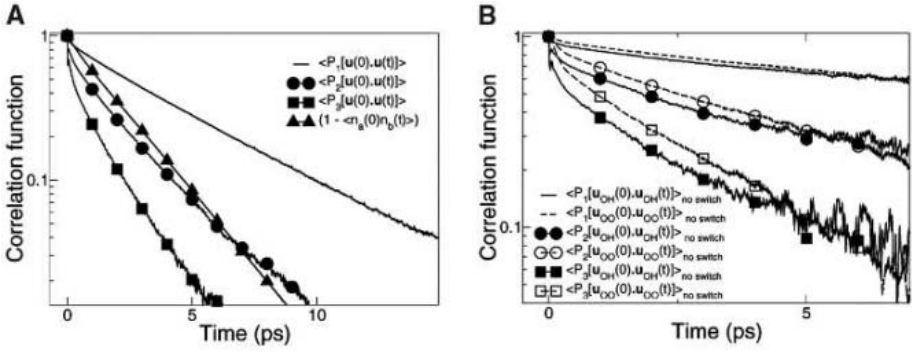

Fig. 4. Orientational and H-bond correlation functions. (**A**) The orientation correlation functions $\langle P_n[\mathbf{u}_{OH}(0)\cdot\mathbf{u}_{OH}(t)]\rangle$, where P_n is the n^{th} order Legendre polynomial and $\mathbf{u}_{OH}$ the rotating OH bond direction, are calculated with the ensemble of all OH bonds from MD simulation. The slope of the $[1 - \langle n_a(0)\, n_b(t)\rangle]$ correlation function provides the H-bond switching rate; the extracted time for a completed switch is longer than that associated with transient H-bond breaking and making (*5*). (**B**) Orientational correlation functions calculated during the time intervals between two successive H-bond switches, for the OH direction and for the OO direction.

Table 1. Comparison of orientational relaxation times from different sources. τ_D is the Debye relaxation time. The τ_n times (excluding τ_D) were extracted from the present MD simulations by an exponential fit of the long-time component of the correlation function (Fig. 4), which eliminates the fast librational decay that is described by neither the jump nor the diffusive models. The single molecule relaxation time τ_1 cannot be directly determined experimentally but can be inferred by (approximate) dielectric relaxation theory models from τ_D, which include collective motions. Depending on the model used, a τ_1 value between 2 and 7.5 ps can be supported (*2*, *29*, *30*); to our knowledge, the τ_3 time for water has not yet been measured (*27*).

Source of τ values	τ_D (ps)	τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	τ_1/τ_2	τ_1/τ_3
Molecular dynamics	9.7*	4.3	2.0	1.3	2.1	3.3
OH diffusion ($D_{OH} = 0.45$ ps^{-1})	–	1.1	0.4	0.2	3.0	6.0
Jump model ($\theta_0 = 60°$, $\tau_0 = 1.8$ ps)	–	5.4	2.2	1.6	2.4	3.4
OO reorientation between switches	–	15.5	5.2	2.8	3.0	5.5
Extended jump model (Eq. 3)	–	4.0	1.6	1.0	2.5	4.0
Experiment	8.5†	2–7.5‡	1.7–2.6§	–	–	–

*(*18*) †(*17*) ‡(*2*, *29*, *30*) §(*12*, *13*, *15*, *16*)

Examination of the τ_1/τ_3 ratio provides an even clearer picture; this ratio is calculated to be 3.3 from the MD simulations, in good agreement with the extended jump model prediction of 4.0, whereas the purely diffusive model yields a value of 6 (*27*).

Therefore the extended jump model, whose parameters are determined in the accompanying simulations, is shown to be fully consistent with the experimental reorientation times and is clearly supported by MD simulations. These results thus call for a reinterpretation of the many experimental data for which water rotation is assumed to be purely diffusive in character.

Further confirmation of the molecular mechanism presented here could emerge from the resolution of the remaining controversial issues for water reorientation, such as the experimental isotope effect in the reorientation times (*15*), and a possible laser OH excitation frequency dependence of the reorientation times (*12*, *13*, *15*) and angular displacement (*28*).

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The Role of Pair Dispersion in Turbulent Flow

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Mixing and transport in turbulent flows—which have strong local concentration fluctuations—are essential in many natural and industrial systems including reactions in chemical mixers, combustion in engines and burners, droplet formation in warm clouds, and biological odor detection and chemotaxis. Local concentration fluctuations, in turn, are intimately tied to the problem of the separation of pairs of fluid elements. We have measured this separation rate in an intensely turbulent laboratory flow and have found, in quantitative agreement with the seminal predictions of Batchelor, that the initial separation of the pair plays an important role in the subsequent spreading of the fluid elements. These results have surprising consequences for the decay of concentration fluctuations and have applications to biological and chemical systems.

Turbulent mixing of liquids and gasses is ubiquitous in nature (*1*); it is the basis of all industrial fluid mixing processes, and it determines the spread of pollutants or bioagents in the atmosphere (*2*) and oceans (*3*). Biological organisms in marine ecosystems also exploit it for their survival (*4*–*6*). A crucial component of turbulent mixing is the fluctuation of local concentration. The rate of destruction of ozone in the atmosphere, for example, is largely determined by these fluctuations rather than by the mean concentration (*7*), as is the toxicity of gas leaks or air pollution. It is natural to relate these concentration fluctuations to the separation of two nearby fluid elements; i.e., pair dispersion (*8*, *9*).

In a quiescent fluid, the relative dispersion of two fluid elements (or tracer particles) is dominated by diffusion. The particles undergo Brownian motion, and the mean square separation between them grows linearly in time. In a turbulent flow, however, if the two particles are separated by distances smaller than the characteristic size of the largest eddies in the flow, they will separate faster (superdiffusively). At large separation times and distances, the local correlations responsible for the superdiffusive separation will no longer be present, and, on average, the relative dispersion will again be linear in time.

Despite almost 80 years of scientific inquiry into relative dispersion (*2*, *9*–*17*), no clear experimental verification of the theoretical predictions has emerged. One critical unresolved question is the extent to which the initial separation of the fluid particles influences their subsequent motion. Our measurements in a laboratory water flow (*18*, *19*) in very intense turbulence suggest that the initial separation remains important for all but the most violent flows on Earth. This observation has consequences for such varied problems as pollution control; combustion modeling; hazardous chemical control; and even the understanding of how animals locate food, predators, and mates (*5*, *6*).

We measured relative dispersion in a water flow at high turbulence levels by using optical particle tracking. This technique has been used for a number of years in turbulence research (*13*, *20*) but was limited to the measurement of low–turbulence level flows, because tracer-particle motions must be resolved over times comparable to the smallest time scale of the turbulence [i.e., the Kolmogorov time scale $\tau_\eta = (\nu/\varepsilon)^{1/2}$, where ν is the kinematic viscosity and ε is the energy dissipation rate per unit mass]. In intense turbulence, these times are often very small. The turbulence level is generally quantified by the Reynolds number, which measures the ratio of the nonlinear inertial forces to the linear viscous forces. Here we report the Reynolds number based on the Taylor microscale, $R_\lambda = \sqrt{(15u'L/\nu)}$,

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where u' is the root mean square (rms) velocity of the turbulent fluctuations and L is the largest length scale of the turbulence. In our water flow at $R_\lambda = 815$, which is the highest Reynolds number reported in this work, $\tau_\eta = 0.54$ ms; therefore, very fast detectors must be used to resolve the fine structure of the flow. Previously, by using silicon strip detectors from high-energy physics experiments (*18*, *19*), we extended the particle tracking technique to flows with high turbulence levels. Such detectors, however, are unsuitable for measuring the statistics of many tracer particles at once. We therefore used three Phantom v7.1 digital cameras from Vision Research, Inc. (Wayne, NJ), which record 27,000 pictures per second at a resolution of 256 × 256 pixels (Fig. 1A). This camera system can be used to track several hundred particles at once (*21*). An example of two such simultaneously measured particle tracks is shown in Fig. 1B.

We generated turbulence between coaxial counter-rotating baffled disks in a closed chamber with a volume of approximately 0.1 m^3 (Fig. 1A). We made measurements in a subvolume of roughly 5 × 5 × 5 cm^3 in the center of the tank, where the mean flow is statistically zero. Polystyrene tracer particles 25 μm in diameter, comparable to the Kolmogorov length scale $\eta = (\nu^3/\varepsilon)^{1/4}$, which is the smallest scale of the turbulence, were illuminated by two frequency-doubled, pulsed Nd–yttrium-aluminum-garnet (Nd:YAG) lasers, with a combined power of roughly 150 W. The particle positions were measured with a precision of roughly 0.1 pixels (*21*), corresponding to about 20 μm in the flow. Further description of this flow has been reported previously (*18*, *19*).

By analyzing our measured particle tracks, we investigated the time evolution of the mean square separation between two fluid elements. Predictions for the superdiffusivity of this pair dispersion in turbulence date back to 1926, when Richardson (*10*) suggested that it should grow in time as t^3. By applying Kolmogorov's scaling theory (*22*), Obukhov (*23*) specified that in the inertial range of turbulence, where the only relevant flow parameter is the energy dissipation rate per unit mass ε, the pair dispersion should grow as $g\varepsilon t^3$, where g is a universal constant. Batchelor (*11*) refined this work, predicting that the mean square separation should grow as t^2 for times shorter than a characteristic timescale t_0, which depends on the initial separation of the pair.

By defining $\Delta(t)$ as the separation of two fluid elements at time t and defining Δ_0 as the initial separation between the fluid elements, Batchelor predicted that for Δ_0 in the inertial range

$$\left\langle \left[\vec{\Delta}(t) - \vec{\Delta}_0\right]^2 \right\rangle = \frac{11}{3} C_2 (\varepsilon \Delta_0)^{2/3} t^2 \quad \text{for } t < t_0 = \left(\frac{\Delta_0^2}{\varepsilon}\right)^{1/3} \tag{1}$$

where C_2 is the universal constant in the inertial range scaling law for the Eulerian second-order velocity structure function with a well-known value of approximately 2.13 (*24*). In the classical cascade model of turbulence, t_0 may be identified as the time for which the two fluid elements "remember" their initial relative velocity as they move in the same eddy of size Δ_0. At times on the order of t_0, this eddy breaks up, and the growth of the pair separation is expected to undergo a transition to Richardson-Obukhov scaling.

To distinguish between Batchelor and Richardson-Obukhov scaling, the inertial range must be large, so that there will be a large separation between the eddy turnover time T_L and the Kolmogorov time τ_η. To achieve such a wide range of scales, the turbulence level must be high because $R_\lambda \sim (T_L/\tau_\eta)$. Based on evidence from direct numerical simulation (*25*), a turbulence level of at least $R_\lambda = 600$ to 700 is required to see true inertial range scaling of a Lagrangian quantity such as relative dispersion. Previous experimental and computational studies of dispersion have been limited by their low turbulence levels ($R_\lambda < 300$) (*12*–*15*, *17*) and have not been conclusive. High turbulence levels are obtained in kinematic simulation models (*16*), but such models may not be suited to the pair dispersion problem (*26*).

Figure 2 shows measurements of relative dispersion for turbulence levels up to $R_\lambda = 815$. We found that for experimentally accessible initial separations, our data scales as t^2 for more than two decades in time, with no hint of classical Richardson-Obukhov t^3 scaling. This behavior holds throughout the entire

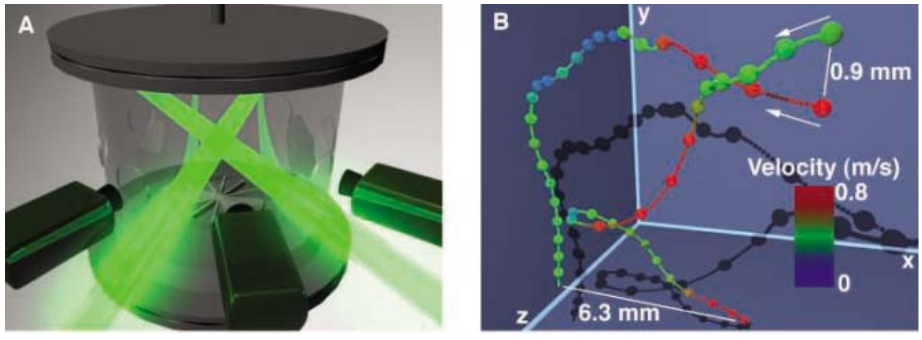

Fig. 1. (**A**) Sketch of the experimental setup. Three high-speed cameras were used to record the three-dimensional tracks of tracer particles in intense turbulence. The particles were illuminated by two high-power lasers. (**B**) A pair of measured particle trajectories at $R_\lambda = 690$. The small spheres mark every other measured position of the particles and are separated by 0.074 ms ($\approx\tau_\eta/13$) in time; the large spheres mark every 30th position. The color of the spheres indicates the magnitude of each particle's absolute velocity in units of m/s. The particles enter the measurement volume as indicated by the arrows and separate under the influence of the turbulence.

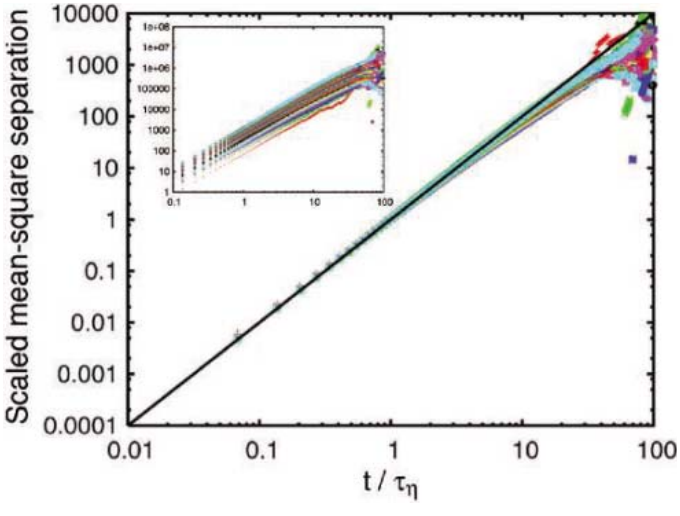

Fig. 2. Evolution of the mean square particle separation. The mean square separation between particle pairs is plotted against time for 50 different initial separations at a turbulence level of $R_\lambda = 815$, with the time axis normalized by the Kolmogorov scales. Each curve represents a bin of initial separations 1 mm wide ($\approx 43\eta$), ranging from 0 to 1 mm to 49 to 50 mm. The curves are scaled by the constant $(\frac{11}{3})C_2(\varepsilon\Delta_0)^{2/3}$ (Eq. 1). The data collapse onto a single universal power law. The bold black line is the power law predicted by Batchelor (*11*). Because the smallest Δ_0 measured is not in the inertial range, we do not expect it to scale perfectly as t^2, and indeed it does not scale as well as the larger Δ_0. The inset shows the same curves scaled simply by the Kolmogorov length, for which we see no scale collapse. For both plots, we see no Richardson-Obukhov t^3 scaling.

inertial range, even for large initial separations (up to 70% of the largest length scale of the turbulence). When we scaled our relative dispersion data by the constant predicted by Batchelor, given in Eq. 1, the curves collapsed onto a single t^2 power law. The line drawn in Fig. 2 is $(\frac{11}{3})C_2(\varepsilon\Delta_0)^{2/3}t^2$.

In Fig. 2, where time is plotted in units of τ_η, the data for different initial separations deviate from the t^2 law at times that vary with Δ_0. If, however, we scale time by Batchelor's $t_0 = (\Delta_0^2/\varepsilon)^{1/3}$ (Fig. 3), the data for each initial separation deviate from Batchelor's prediction at the same universal value of roughly 0.1 t_0, irrespective of turbulence level.

For the quantities plotted in Figs. 2 and 3, we see no Richardson-Obukhov t^3 scaling. We also, however, measured other statistics that, dimensionally, should obey the same scaling laws. One such quantity is exit time statistics (*14*). Our measurements of such statistics showed no clear t^3 behavior. Another measure of relative dispersion is shown in Fig. 4, in which we plot $(\langle\Delta^{2/3}\rangle - \Delta_0^{2/3})$ scaled by $\Delta_0^{2/3}$ as a function of t/t_0. For small initial separations for which (T_L/t_0) is of order 10, we see a transition to a scaling law consistent with the Richardson-Obukhov prediction for times greater than roughly t_0, irrespective of turbulence level. For larger initial separations for which (T_L/t_0) is smaller, however, no such scaling is seen, as shown in the inset to Fig. 4. The existence of a transition at times on the order of t_0 shows that the initial separation is an important parameter for relative dispersion and cannot be neglected.

In any practical application of relative dispersion, the initial source will have finite size and therefore have a nonzero Δ_0. Our data show that t_0 accurately quantifies the transition between the Batchelor scaling regime and the Richardson-Obukhov regime. Consequently, a clear t^3 scaling law requires not only a large separation between T_L and τ_η but also a large separation between T_L and t_0. For the initial separations accessible in our experiments, the maximum value of the ratio of (T_L/t_0) was of order 10, with no fully developed t^3 scaling. In order to apply the Richardson-Obukhov scaling law to a practical situation, then, (T_L/t_0) must be much larger than 10, which implies the necessity of a high turbulence level.

For most flows on Earth, both natural and industrial, the turbulence levels are quite small: typically, $R_\lambda < 1000$. Very turbulent atmospheric flows, such as warm clouds or the atmospheric boundary layer (*27*), have turbulence levels of about $R_\lambda \sim 10^4$. Even the most violent flows on Earth, such as plinian volcanic eruptions, have similar turbulence levels. If we consider a pair of particles with an initial separation of roughly 1 m, such as might be found in the smokestack of an industrial plant, for a turbulence level of $R_\lambda \sim 10^4$, (T_L/t_0) is only about 30, assuming typical atmospheric flow parameters (*28*).

An important consequence of these results is that in almost all flows with industrial or biological significance, the initial separation Δ_0 will influence the subsequent spreading of the two fluid elements throughout the entire period of their turbulent superdiffusive separation. This can explain, for example, measurements of the decay of the fluctuations of a passive scalar injected into the flow (*29*). This decay became slower as the separation between two sources was increased. These results may, in turn, explain why the spatial arrangement of odor sources plays such an important role in the way crayfish and other crustaceans, as well as organisms both larger and smaller (*6*), navigate their marine environments (*5*).

We observed that Batchelor's prediction is fulfilled for more than two decades in time at high turbulence levels. Although our data may be somewhat contaminated by the inhomogeneity and anisotropy present in our specific flow, the observed scale collapse onto the Batchelor law appears very robust. In addition, we showed that the initial separation of the particle pair remains important in most flows in nature up to times of order t_0, which itself depends on the initial separation. We observed a transition near t_0 only when (T_L/t_0) was of order 10 or larger. Therefore, a large separation between T_L and t_0 is required to see a fully developed Richardson-Obukhov scaling regime, requiring a turbulence level beyond the reach of current experiments and higher than will occur in most practical situations.

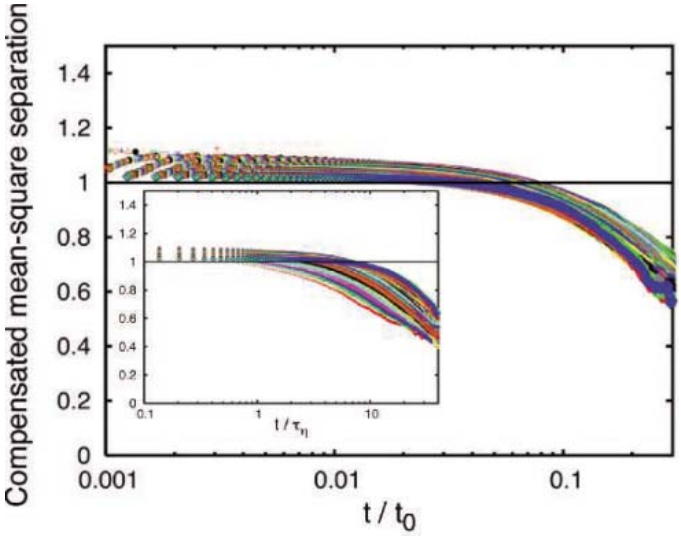

Fig. 3. Mean square separation with time scaled by t_0. The mean square separation at $R_\lambda = 815$ compensated by Batchelor's scaling law (Eq. 1) is plotted against time in units of $t_0 = (\Delta_0^2/\varepsilon)^{1/3}$. Plotted in this way, a plateau corresponds to Batchelor scaling. The inset shows the same compensated data plotted against time and scaled by the Kolmogorov time. The data clearly collapse significantly better with time scaled by t_0. The data begin to deviate from a t^2 power law at a universal time of about 0.1 t_0.

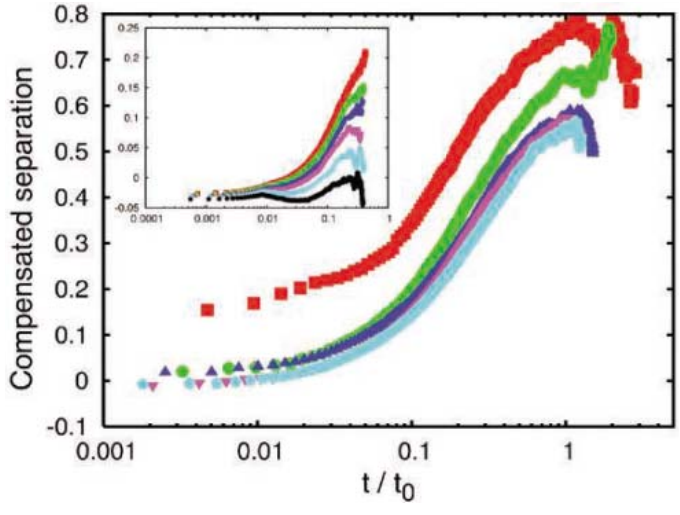

Fig. 4. $[\langle\Delta(t)^{2/3}\rangle - \Delta_0^{2/3}]$ scaled by $\Delta_0^{2/3}$ and compensated by t/t_0. The data are plotted against t/t_0; a plateau denotes Richardson-Obukhov–like scaling. The initial separation increases from 1 mm (≈43η) for the top curve to 5 mm (≈215η) for the bottom curve, and (T_L/t_0) is of order 10. The inset shows the same quantity plotted against t/t_0 for larger initial separations, ranging from 20 mm (≈860η) for the top curve to 30 mm (≈1290η) for the bottom curve.

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Late Quaternary Atmospheric CH_4 Isotope Record Suggests Marine Clathrates Are Stable

Todd Sowers

One explanation for the abrupt increases in atmospheric CH_4, that occurred repeatedly during the last glacial cycle involves clathrate destabalization events. Because marine clathrates have a distinct deuterium/hydrogen (D/H) isotope ratio, any such destabilization event should cause the D/H ratio of atmospheric CH_4 (δD_{CH_4}) to increase. Analyses of air trapped in the ice from the second Greenland ice sheet project show stable and/or decreasing δD_{CH_4} values during the end of the Younger and Older Dryas periods and one stadial period, suggesting that marine clathrates were stable during these abrupt warming episodes. Elevated glacial δD_{CH_4} values may be the result of a lower ratio of net to gross wetland CH_4 emissions and an increase in petroleum-based emissions.

The ice core record of atmospheric CH_4 changes covering the past 650,000 years exhibits two primary frequencies. Over long time scales (greater than 10,000 years) atmospheric CH_4 changes have a substantial amount of variance concentrated in the precessional bandwidth (19,000 and 23,000 years) (*1*, *2*) that is considered to be an integral part of tropical climate throughout the late Pleistocene. One hypothesis that accounts for this observation involves an energized hydrologic cycle during periods of elevated low-latitude insolation. The invigorated hydrologic cycle promotes an increase in wetland extent driving a concomitant increase in CH_4 emissions that raise atmospheric CH_4 levels during warm periods. Embedded within the precession signal are millennial- and century-scale variations that are tightly coupled to Greenland temperature (*3*, *4*). In general, increasing atmospheric CH_4 levels are synchronous with, or slightly lag (by a few decades), the surface temperature increase over Greenland (*5*). Assessing the nature of these abrupt CH_4 events is important for understanding how ecosystems and climate are connected and in estimating the degree to which future CH_4 levels may contribute to changes in Earth's radiation budget.

There are two competing explanations for the abrupt CH_4 increases. One hypothesis holds that the terrestrial biosphere is capable of rapidly increasing CH_4 emissions in response to abrupt changes in the hydrologic cycle that are teleconnected to surface temperatures over Greenland (*3*, *4*). The other explanation involves the sudden release of marine clathrates situated along the continental margin where episodic destabilization events may have been triggered by enhanced ventilation (warming) of upper thermocline waters (*6*). The majority of the released CH_4 ultimately travels across the air-sea interface leading to atmospheric CH_4 increases.

Model estimates of changes in the primary CH_4 sink (tropospheric hydroxyl radical) during the last glacial termination suggest that the observed CH_4 variations must be due in large part to changes in the sources as opposed to changes in the rate of removal (*7*). The isotopic composition of atmospheric CH_4 therefore provides additional information on the relative contribution of the various sources. Variations in the D/H ratio of atmospheric CH_4 (δD_{CH_4}) can be used to infer variable clathrate contributions on the basis of their elevated δD values compared with all terrestrial CH_4 sources (Fig. 1). Methane clathrates within the continental margin sediments are formed almost exclusively by CO_2 reduction or thermal cracking of longer chain hydrocarbons, whereas terrestrial CH_4 emissions are primarily aceticlastic in nature (*8*, *9*). During CO_2 reduction, all the methyl hydrogen atoms come directly from porewater H_2 that is in isotopic equilibrium with the porewater (*10*). The resulting δD_{CH_4} values are lower than the porewater δD_{H_2O} due to a ~180 per mil (‰) biologically induced isotope effect associated with CO_2 reduction (*9*, *11*). Marine clathrate δD_{CH_4} values from 13 nearshore sites scattered throughout the Northern Hemisphere are surprisingly constant (–189 ± 27‰; error is SD) given the diverse nature of the geologic and sedimentologic settings and the varying proportions of microbial and thermogenic CH_4 at each site (*12*, *13*). In contrast, CH_4 production in terrestrial ecosystems is dominated by acetogenesis (acetate fermentation) where three-fourths of the hydrogen atoms in the emitted methane originate from the methyl group associated with the acetate substrate. The remaining hydrogen comes from the local water with the resulting terrestrial δD_{CH_4} values generally ranging from –250 to –380‰, with the local δD_{CH_4} value strongly influenced by the δD of precipitation (*8*, *9*).

An atmospheric δD_{CH_4} record (Fig. 2) was generated from the second Greenland ice sheet project (GISP II) ice core using a previously described technique with an external precision of ±4.2‰ (*14*). The general picture of δD_{CH_4} variations associated with the deglaciation shows a progressive decrease in δD_{CH_4} as the concentration of CH_4 increases, opposite to that predicted by increasing clathrate contributions due to warming associated with the termination. During the last glacial maximum (LGM), δD_{CH_4} values were generally ~5‰ higher than the Bolling/Allerod values [15 to 13 thousand years ago (ka)] and ~20‰ higher than early Holocene values. There are three factors that can be reasonably constrained as contributing to the elevated δD_{CH_4} values during the LGM. All three factors are temperature dependent, so

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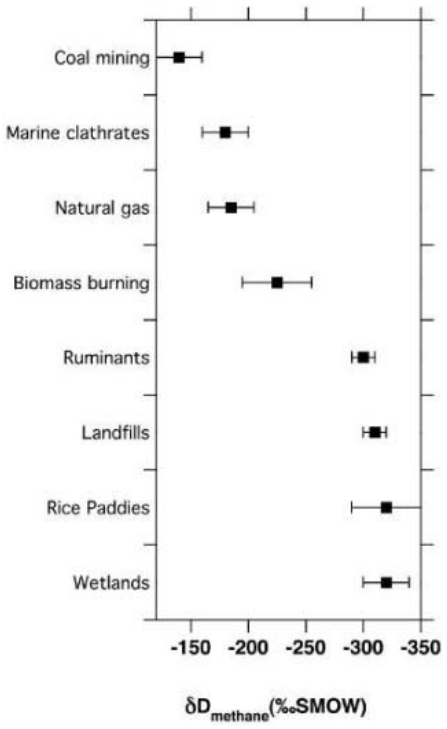

Fig. 1. Characteristic δD_{CH_4} values for various present-day CH_4 sources. All the data except the value for the marine clathrates (*13*) are from (*26*–*28*). Present day atmospheric δD_{CH_4} estimates are ~–90 ± 5‰ (*29*). The enriched atmospheric value is the result of a large KIE (+250‰) associated with the primary sink (tropospheric OH) (*14*). Error bars for each source correspond to the tabulated range of values. SMOW, standard mean ocean water.

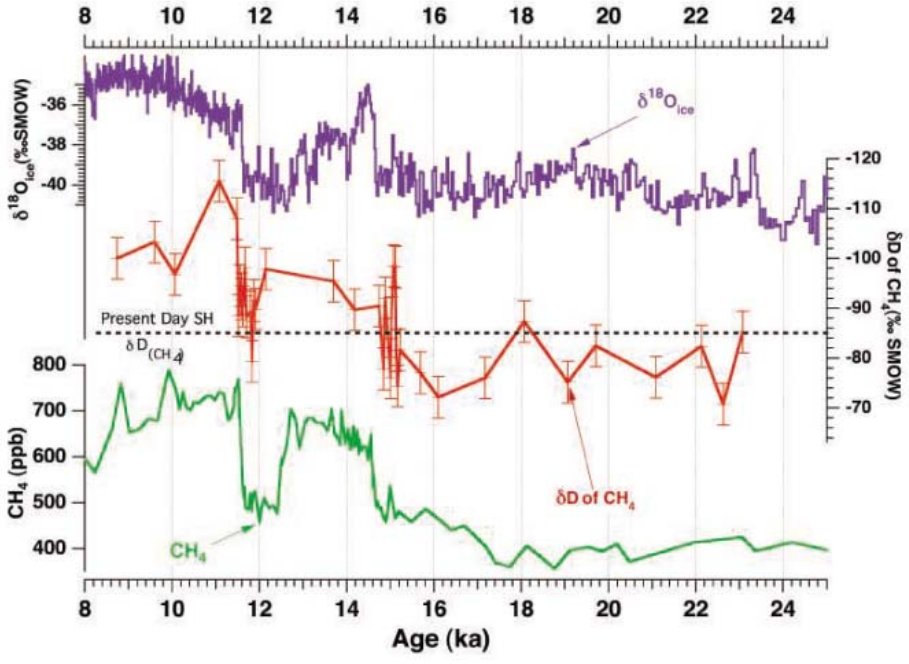

Fig. 2. Results from the last glacial termination as recorded in the GISP II ice core. The upper purple curve is the isotopic temperature (*30*). The red curve is from the current δD_{CH_4} analyses with 1σ (4.2‰) error bars. The bottom CH_4 concentration curve (green) is from Brook *et al.* (*3*). ppb, parts per billion. The increased sample resolution associated with the abrupt CH_4 concentration increases associated with the onset of the Bolling/Allerod and the end of the Younger Dryas periods are shown in expanded view in Fig. 3. The present-day δD_{CH_4} value for the Southern Hemisphere (SH) is shown as a horizontal dashed line for reference.

Table 1. Constrainable factors influencing δD_{CH_4} during the LGM.

	$\Delta\delta D_{CH_4}$ (LGM to Holocene) (‰)	
Factor	CLIMAP SST*	5°C Tropical cooling
10% decrease in C_3/C_4 ratio during LGM†	–1.9	0
KIE for OH oxidation	0	3.4
ΔSea level‡	5.1	0 to –5
Total change	+3.2	3.4 to –1.6

*CLIMAP Climate: Long-Range Investigation, Mapping, and Prediction; SST, sea surface temperature. †Assuming the D/H ratio in C_3 and C_4 plants differs by ~15‰ [C_3 plants have higher δD values (*31*)], then a 10% reduction in the C_3/C_4 ratio of wetland plants during the LGM (*21*) would have raised atmospheric δD_{CH_4} values by ~1.9‰ relative to Holocene values. Assume additional 5°C cooling during LGM yields no change in C_3/C_4 ratio. ‡Seawater δD_{H_2O} during LGM = 7.5‰ standard mean ocean water (*18*). General circulation model simulations suggest little change in δD_{H_2O} precipitation using CLIMAP SST (*19*, *20*) but a slight decrease in δD_{H_2O} precipitation for 5°C tropical cooling (*19*). Finally, assume $\delta D_{CH_4}/\delta D_{H_2O}$ = 0.675 (*8*).

estimates have been made on the basis of two different tropical LGM temperature estimates (Table 1). First, colder temperatures during this period would have increased atmospheric δD_{CH_4} through the temperature-dependent kinetic isotope effect (KIE) associated with the primary removal process, OH oxidation in the troposphere. The magnitude of this effect is +3.4‰ on the basis of the laboratory-determined temperature dependence (*15*), assuming tropospheric temperatures were 5°C colder during the LGM. Secondly, δD changes in mean ocean water arising from changes in continental ice volume impart a direct effect on atmospheric δD_{CH_4} by altering the δD of porewater H_2 that is utilized by CO_2 reducing methanogens (oceanic and terrestrial). A less direct effect occurs as the oceanic δD_{H_2O} change is propagated through the hydrologic cycle and incorporated in terrestrial organic hydrocarbons (*16*, *17*), the primary substrate for the fermentative methanogens. The mean ocean δD_{H_2O} change [7.5‰ (*18*)] is transferred through the LGM global hydrologic cycle (*19*, *20*), causing changes in δD_{CH_4} values for terrestrial CH_4 that range from –5 to 5‰, depending on the assumed LGM tropical temperatures (*8*, *9*). Finally, a 10% decrease in the ratio of C_3- to C_4-type plants during the LGM (*21*) would have lowered atmospheric δD_{CH_4} values by 0 to 1.9‰ relative to Holocene values. Together these three factors account for a small portion of the observed 20‰ δD_{CH_4} shift between the LGM and early Holocene, implying that other factors must be considered.

There are at least three additional factors contributing to the atmospheric δD_{CH_4} change associated with the termination that are difficult to quantify. First, elevated δD_{CH_4} values during the LGM may be the result of a decrease in the ratio of net to gross (N/G) CH_4 production. It has been fairly well documented through inhibitor studies that as much as 50% of the CH_4 produced at depth in soils is consumed by microbially mediated methane oxidation near the soil-atmosphere interface (*22*). The δD_{CH_4} values for the emitted CH_4 are strongly dependent on the N/G ratio because of the large KIE associated with methane oxidation [–95 to –285‰ (*23*)]. For example, lowering N/G by 11% during the LGM (with the δD_{CH_4} of gross CH_4 assigned as –300‰ and the KIE for methane oxidation as –95‰) would raise atmospheric δD_{CH_4} by 10‰. The sense of this change is consistent with observations that the methane-producing communities are more sensitive to temperature changes than methane-oxidizing communities (*24*).

Two additional factors contributing to the elevated δD_{CH_4} values during the LGM involve an increase in the relative proportion

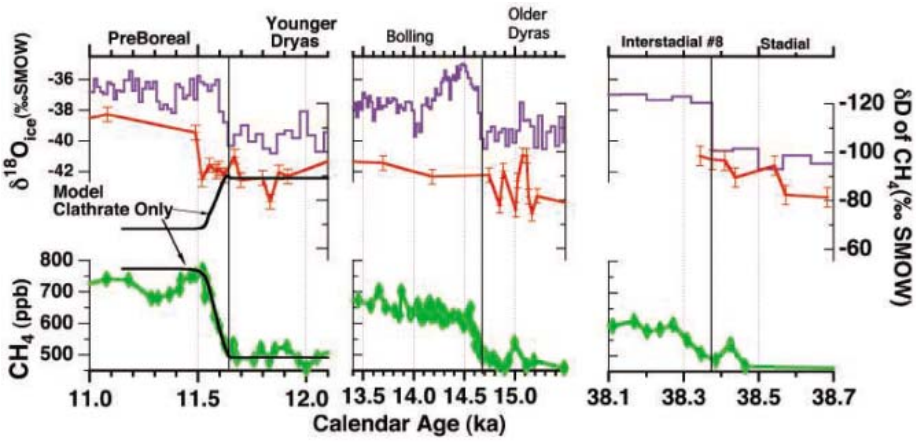

Fig. 3. Expanded views of three abrupt CH_4 concentration events recorded in the GISP II ice core. The isotopic temperature record (*30*) and atmospheric CH_4 concentration record (*3*) are plotted for reference. The red curves are from the current δD_{CH_4} analyses with 1σ errors at each measured depth. The results from the one-box clathrate-only model are shown for the Younger Dryas simulation with black curves. All data in all three panels are plotted on the same *y* axes for comparison.

of petroleum-based and/or biomass-burning CH_4 emissions, both of which have elevated D/H ratios (Fig. 1). Model simulations of biomass burning, however, suggest lowered CH_4 emissions during the LGM (*25*). If, as recently suggested (*12*, *13*), CH_4 from petroleum seeps contributed a larger proportion of global sources during the LGM compared with early Holocene periods, then we would expect higher atmospheric δD_{CH_4} values during the LGM. Assuming global CH_4 emissions during the LGM were 111 Tg/year (*3*) and the characteristic δD_{CH_4} value for the terrestrial biosphere was –300‰, then a 10‰ δD_{CH_4} signal can be accounted for by increasing the fraction of CH_4 emissions based on petroleum and/or biomass burning by 9% during the LGM (compared with early Holocene emissions).

The high-resolution δD_{CH_4} records during the end of the Younger and Older Dryas periods (11.5 and 14.7 ka, respectively) and the onset of interstadial 8 (IS8) (38.5 to 38 ka) provide important constraints for assessing clathrate stability during these periods (Fig. 3). With the exception of one short period of increasing δD_{CH_4} between 15.2 and 15.0 ka, the atmospheric δD_{CH_4} record from GISP II shows relatively stable or slightly decreasing δD_{CH_4} values during periods of increasing CH_4 concentration. This trend in not consistent with either a gradual or an episodic release of clathrates, suggesting that marine clathrates were stable throughout the last glacial termination as well as during periods of abrupt warming.

To estimate the magnitude of the atmospheric δD_{CH_4} shift associated with a hypothetical clathrate destabilization event, a simple one-box model of the atmosphere was developed using the CH_4 concentration history from the end of the Younger Dryas period to constrain total CH_4 emissions (*3*). The model consists of two sources and a single sink term. Terrestrial CH_4 emissions, the lifetime of atmospheric CH_4, the δD_{CH_4} value for terrestrial CH_4 emissions (–300‰), and the KIE associated with the sink (+165‰) were all held constant throughout the simulation. Then, beginning at model year 11.64 ka, we introduced clathrate-derived CH_4 ($\delta D_{CH_4} = -189‰$) at a rate of 0.8 Tg/year for the next 100 model years, after which clathrate emissions were held constant at 80 Tg/year. The model predicted evolution of CH_4 concentration and δD_{CH_4} are included in Fig. 3 for comparison with the GISP II data from the Younger Dryas. Assuming clathrate CH_4 was the only new CH_4 source at the end of the Younger Dryas, the predicted δD_{CH_4} change was +21‰. The relatively constant δD_{CH_4} values throughout the transition to elevated CH_4 levels suggest little change in the relative proportion of all individual emissions with near-constant characteristic δD_{CH_4} values.

The transition from the Older Dryas to Bolling period (15 to 14 ka) provides a very different view of the factors influencing δD_{CH_4} (Fig. 3). During the 300-year period immediately preceding the abrupt increase in atmospheric CH_4 loading, δD_{CH_4} initially decreases by 15‰ followed by a rapid 10‰ increase, during which time atmospheric CH_4 levels remained effectively constant. Obviously, many more data are needed to document this oscillation but, with the limited data in hand, it appears that a rapid shift in the characteristic δD_{CH_4} values of various sources is needed in the absence of substantial global emission changes during a period of relative climate stability.

The general trend of decreasing δD_{CH_4} throughout the termination, combined with relatively stable δD_{CH_4} values during periods of rapidly increasing CH_4, suggests that marine clathrates are stable during this period and specifically during abrupt warming events. The elevated LGM δD_{CH_4} values are likely to be related to a number of factors, the most important being decreased N/G ratios and an increase in petroleum-based CH_4 emissions during the glacial period. Further insight into these factors will derive from future measurements of $\delta^{13}C_{CH_4}$ that are in progress.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/838/DC1
Materials and Methods
Table S1
References

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The Spatial Extent of 20th-Century Warmth in the Context of the Past 1200 Years

Timothy J. Osborn* and Keith R. Briffa

Periods of widespread warmth or cold are identified by positive or negative deviations that are synchronous across a number of temperature-sensitive proxy records drawn from the Northern Hemisphere. The most significant and longest duration feature during the last 1200 years is the geographical extent of warmth in the middle to late 20th century. Positive anomalies during 890 to 1170 and negative anomalies during 1580 to 1850 are consistent with the concepts of a Medieval Warm Period and a Little Ice Age, but comparison with instrumental temperatures shows the spatial extent of recent warmth to be of greater significance than that during the medieval period.

Establishing the history of hemispheric or global temperatures is one fundamental requirement for identifying the contributions of different natural forcings to past climate variability and for quantifying the significance of greenhouse gas–induced warming during the 20th century (*1*–*3*). A number of studies (*1*, *4*–*14*) have selected, combined, and then calibrated multiple climate proxy records to provide assessments of temperature variability on near-hemispheric scales for the past few hundred to two thousand years. Both individually and taken as a whole, these reconstructions have been used to support the conclusion that it is likely that the late 20th century was the warmest period during the past millennium (*15*, *16*) or longer (*11*, *12*, *17*) in the Northern Hemisphere (NH).

Assessing whether these recent temperatures are unprecedented depends on comparing the recent instrumental temperature record with the earlier proxy-based temperature reconstructions. Quantitative calibration of the reconstructions is essential, and the comparison with the instrumental record is only valid if it takes account of the uncertainties associated with interpreting a specific reconstruction as an estimate of the "actual" temperature. Of the studies cited above, some do not provide reconstructions that cover the whole of the millennium (*1*, *8*, *13*, *14*), whereas some others either do not estimate reconstruction uncertainty at all (*4*, *6*, *7*, *10*) [note that reconstruction uncertainty for (*4*) was later estimated by (*16*)] or do not estimate reconstruction uncertainty in a way that is appropriate for assessing the significance of very late 20th-century warmth (*9*, *12*); see (*18*). There are, therefore, currently only three studies (*5*, *11*, *16*) that allow a formal quantitative comparison of late 20th-century instrumental temperatures against reconstructed temperatures for the past 1000 years or more. These three studies all found that recent temperatures are above the 95% uncertainty range estimated to be appropriate for their reconstructions of all earlier temperatures.

The published uncertainties were calculated from the regression residuals during the calibration period (*19*) and probably underestimate the true uncertainties, because additional unquantified error might arise (i) from nonstationarities in proxy-climate (*5*), interseason, (*20*) or land-ocean (*21*) relationships; (ii) from the use of the same period to select and calibrate temperature-sensitive proxies as well as for estimating uncertainty; or (iii) from biases inherent in the calibration method (*22*) [but see (*23*)]. For these reasons, the Intergovernmental Panel on Climate Change (*15*) correctly judged that the conclusion that recent warmth is unprecedented in the context of the past 1000 years could be made with only 66 to 90% confidence, despite recent temperatures exceeding the published 95% uncertainty ranges of all earlier reconstructed values (*5*, *11*, *16*).

A separate analysis on a record-by-record basis of many environmental and climate proxies concluded that the 20th century was probably not the warmest of the last millennium (*24*). This study has been criticized (*25*) for its lack of rigour in assessing whether the proxies used are useful indicators of temperature, for not distinguishing between regionally restricted anomalies and hemispheric-scale warmth, and for providing no calibration or uncertainty estimates that would enable comparison with late 20th-century temperatures. Here, we investigated whether a more carefully designed assessment of proxy records on an individual basis supports the conclusion that recent NH temperatures are unusual in the context provided by these records. We only used proxy records that are positively correlated with their local temperature observations, and, critically, periods with synchronous "warm" or "cold" anomalies in many proxies were used to infer hemispheric-scale climate anomalies as distinct from asynchronous warming or cooling in different regions. This restricts the analysis to those proxy records that are accurately dated. Analysis of synchronous anomalies in a number of independent records is indicative of the geographical extent of anomalous temperatures.

The criteria that proxy records must be well dated and sufficiently resolved and up to date to allow a quantitative comparison with instrumental temperatures eliminates many of the records used by (*24*). Here, we have pooled all of the proxy records used by (*9*) and (*25*) with those high-resolution NH series used by (*11*), then removed duplicates and those that were not positively correlated with their local temperature observations (*26*). Table S1 provides details of the 14 proxy series used here.

The 14 proxy series were each smoothed to remove variations on time scales shorter than 20 years and then "normalized" (*26*) to have zero mean and unit standard deviation (SD) over the full period of analysis, 800 to 1995. The analysis was not continued beyond 1995 because fewer than five of the proxy series were available after 1995. Individually, these records present a relatively complex picture of variability over the last 1200 years (Fig. 1). Although there are periods with coherent changes across a number of records, the implications of such periods cannot be quantified or adequately intercompared by a purely visual analysis.

The proxy records were analyzed simply by counting the fraction of those series that have data in any given year whose smoothed and normalized values exceed certain thresholds (*26*). The thresholds used are the series mean and 1 or 2 SD above or below the mean. The differences between pairs of these fractional exceedance time series were also analyzed (i.e., the fraction of records at least 1 SD above the mean minus the fraction that are at least 1 SD below the mean). All proxies are given equal weight in this analysis.

The statistical significance of the difference time series was established by using a Monte Carlo approach (*26*). The values in each smoothed proxy time series were shifted by a randomly chosen number of years, with values that were shifted beyond the end date of the record cycled back to the start date of the record. The random shifting of the records destroys the calendar alignment between values in different proxy records but maintains the autocorrelation structure of the individual series. The exceedances, differences, and filtering were recalculated from the randomly shifted records, with the entire procedure repeated 10,000 times to build up a distribution of possible values.

Although there are some individual years when the smoothed records are all positive or all negative, these are not sustained sufficiently long for the 20-year smoothed counts of the fractional exceedances to reach one (Fig. 2). Nevertheless, almost all of the series are posi-

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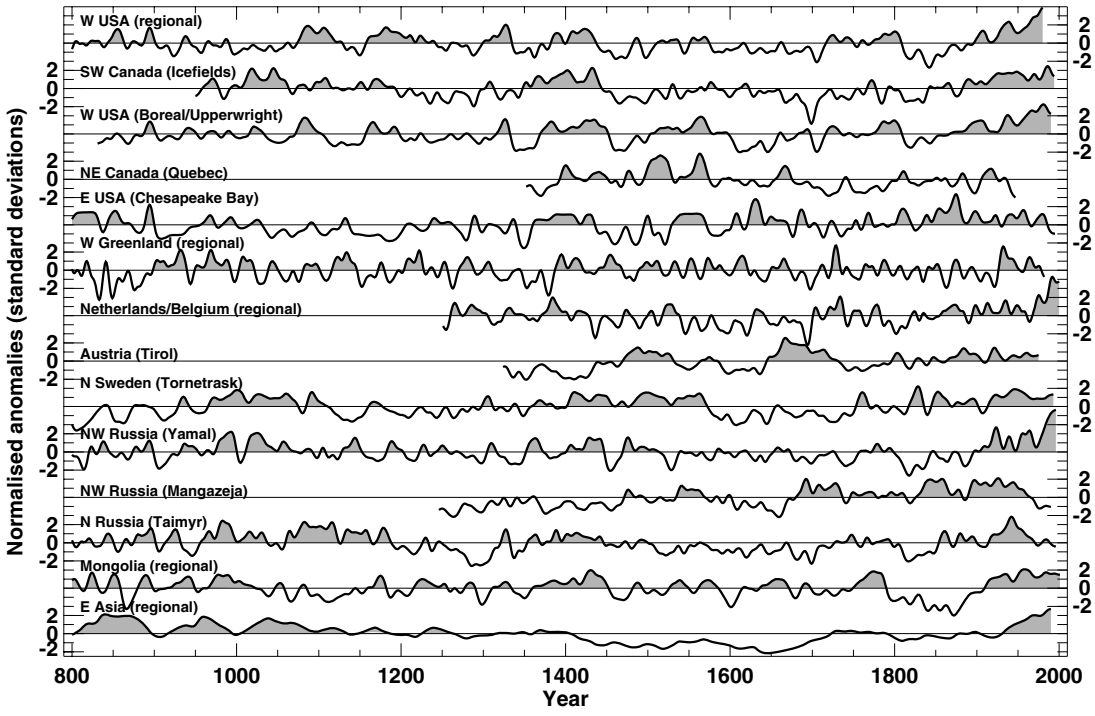

Fig. 1. The 14 temperature-related proxy records used in this study, filtered to remove variations on time scales less than 20 years and then normalized to have zero mean and unit standard deviation during the period from 800 to 1995 [with adjustments made to the shorter records (*26*)].

tive for much of the 20th century, peaking in number between 1935 and 1955. At the end of the analysis period in the early 1990s, 70% of the records have positive values whereas 30% are negative. Of the 70% with positive values, all exceed 1 SD above their respective mean and half exceed 2 SD above their mean, whereas throughout the 20th century the number of records more than 1 SD below their mean is nearly zero. The pre-20th-century periods when no series fall below –1 SD occur mostly between 890 and 1100, and at these times typically 30 to 40% of series simultaneously exceed +1 SD (light red shading in Fig. 2). The periods when no series exceed +1 SD or at least 30% fall below –1 SD (light blue shading) occur mostly between 1230 and 1360 or 1575 and 1840.

The fraction of positive records and the fraction of negative records do, of course, provide the same information, and thus their difference (Fig. 3A) has the same shape, with the highest value being reached in the mid-20th century and the lowest in the first half of the 17th century. These values far exceed even the 1st and 99th percentiles of the Monte Carlo results, providing support for a climate signal that deviates significantly from the overall mean state. The significance levels in all three panels of Fig. 3 vary through time according to the number of series that are available in each year; the greatest number are available between 1352 and 1947, and the detectability of significant anomalies is enhanced during this period (*27*). The value (Fig. 3A) at the very end of the analysis period would have exceeded the 95th percentile if it had occurred when the full set of proxy records were available, but because

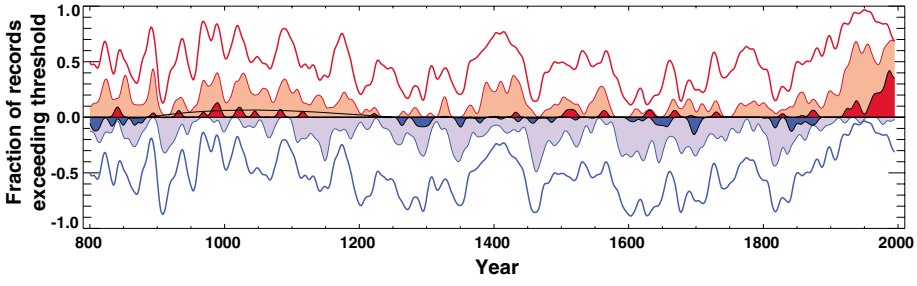

Fig. 2. Fraction of the records available in each year that have normalized values > 0 (red line), > 1 (light red shading), > 2 (dark red shading), < 0 (blue line), < –1 (light blue shading), and < –2 (dark blue shading), with the latter three series multiplied by –1 before plotting. The series are shown from 800 to 1995 and have been filtered to remove variations on time scales less than 20 years.

of the widening of the percentiles after some series end in the late 20th century, the 1995 value falls on the 90th percentile. Significant positive deviations also occur at intervals between 890 and 1170 and near to 1400, whereas significant negative deviations occur between 1200 and 1350, near 1460, and in the late 1600s and the early 1800s.

A similar picture emerges when considering the difference between counts of records more than 1 SD above and below their means (Fig. 3B), except that the values at the end of the analysis period (early 1990s) are similar to those in the mid-20th century. The 20th century is the most anomalous period in the record, with values far exceeding both the 99th percentile of the Monte Carlo results and all earlier values, right through to 1995.

The difference between the high and low 2 SD exceedances (Fig. 3C) shows only small deviations from zero throughout the analysis period except during the late 20th century, which exceeds all other periods, including the mid-20th century. This conclusion relies on the very small number of records whose values depart by more than 2 SD from their means at this time and is more sensitive to the selection of the proxy records than the results obtained using the less extreme thresholds (Fig. 3, A and B). There are earlier intervals with predominantly positive or negative deviations, but very few of these periods lie outside the range expected by chance.

Direct comparison of these results with instrumental temperatures is not possible because the latter records cannot be normalized over the 800-to-1995 period. The proxy data analysis was instead repeated with each series normalized over the 1856-to-1995 period of overlap with the instrumental temperatures. The difference between the fraction of proxy records with positive versus negative anomalies, relative to the shorter 1856-to-1995 reference period mean (Fig. 3D), shows a similar time evolution as the longer 800-to-1995 reference period re-

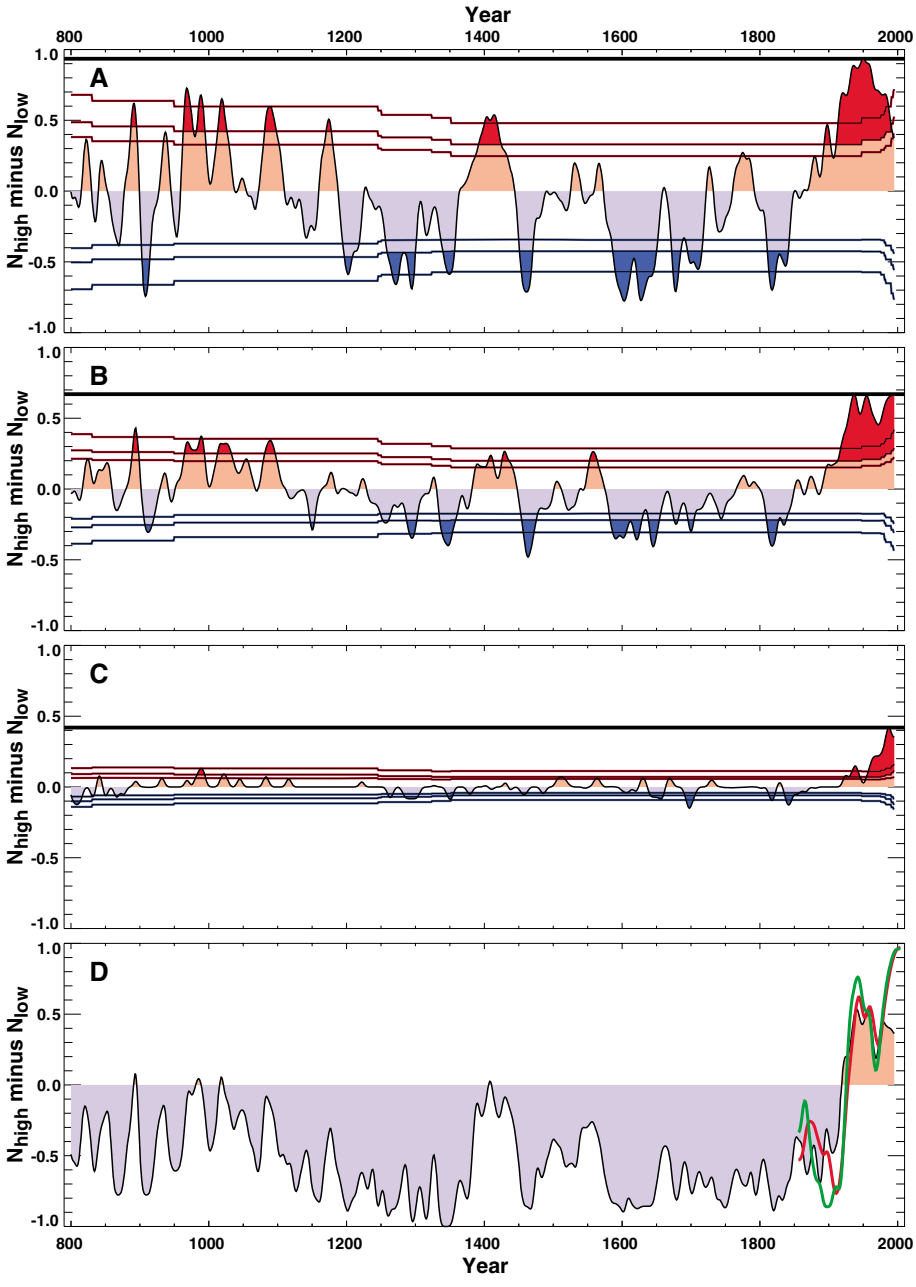

Fig. 3. Difference between the fraction of the records available in each year that have normalized values (**A**) > 0 and < 0, (**B**) > 1 and < −1, (**C**) > 2 and < −2, and (**D**) as (A) but using a shorter (1865 to 1995) reference period for normalization. The difference series are shown for 800 to 1995 and have been filtered to remove variations on time scales less than 20 years. Zero indicates that the number of series exceeding the upper threshold equals those with values below the lower threshold. In (A) to (C), the highest values achieved by each difference series are indicated by the horizontal black lines; the red and blue lines show the 99th, 95th, 90th, 10th, 5th, and 1st percentiles of distributions obtained by repeating the analysis 10,000 times with each proxy time series shifted randomly in time; and dark red and blue shading indicates times when the difference series exceeds the 95th or 5th percentiles. In (D), results based on annual-mean instrumental temperatures from grid boxes throughout the NH (red curve) or only in regions close to the proxy records (green curve) are shown for 1856 to 2004 (also normalized over the period from 1856 to 1995).

sults (Fig. 3A), although shifted vertically, of course.

The same analysis (filtering, normalizing, and counting values that exceed the various thresholds) was also applied to annual mean instrumental temperatures (*28*) from all grid boxes with data available in the NH or alternatively only to grid boxes close to the locations of the 14 proxy records. The similarity of the two instrumental temperature curves (Fig. 3D) indicates that the 14 proxy sites provide sufficient coverage to estimate the NH temperature behavior and that the limiting factor is likely to be the skill with which each proxy chronicles its local temperature variations. The instrumental temperature results show a close correspondence with the proxy records, particularly for the early 20th century increase and the variations during the 1930 to 1975 period. Each of the proxy records undoubtedly includes some variance that is unrelated to local temperature variations, and the characteristics of this "noise" determine the extent to which the signal shown by the counts of threshold exceedances and their differences will be expressed. The slight underestimation by the proxy results of the early 20th century rise and the absence of a further increase at the end of the records could both be examples of the expected consequences of noise in the proxy records. Virtually every grid-box instrumental temperature series in the NH exceeds its 1856 to 1995 mean level by the end of these records in 2004.

Similar results are obtained for the 1 or 2 SD thresholds, but these results are not shown here because estimating the SD of 20-year smoothed time series using this relatively short reference period (140 years) results in larger uncertainty in the counts of exceedances than for the results shown in Fig. 3D.

The multidecadal intervals (Figs. 2 and 3) with significantly widespread positive anomalies between 890 and 1170 and significantly widespread low proxy values between 1200 and 1850 (interspersed by periods with high or near-zero anomalies) provide support for the concepts of anomalous medieval (*29*) and Little Ice Age (*30*) periods (particularly from the late 1500s to the mid-1800s), although they are clearly discontinuous in time (with consequently ill-defined dates of onset and termination) and geographically restricted. The 20th century is the most anomalous interval in the entire analysis period, with highly significant occurrences of positive anomalies and positive extremes in the proxy records. These results are not dependent on the inclusion of specific individual proxies or the choice of reference period (figs. S2 to S6).

The approach used here is complementary to those studies that combined multiple proxy records into a calibrated time series of past large-scale or NH mean temperatures. By analyzing the raw proxy records themselves, some of the issues associated with the combination and calibration of records have been avoided [e.g., choice of optimum regional or seasonal temperature (*13*, *20*); sensitivity to calibration period, time scale, and regression method (*13*, *31*); and potential bias in some regression methods (*22*, *23*)]. In avoiding these issues, however, we have been compelled, thus far, to restrict the interpretation of our results to periods of "unusually high or low" proxy values rather than as indicative of "warm" or "cool" periods.

There is support, however, for interpreting the results of Figs. 2 and 3 as indicators of NH temperature: (i) there is strong evidence for

a common environmental signal in the proxy records because the counts of simultaneous threshold exceedances lie well outside the ranges obtained by the Monte Carlo simulations; (ii) this environmental signal is most likely to be a climate signal because the departures from the Monte Carlo ranges do not just occur during the twentieth century, when very widespread nonclimatic anthropogenic disturbances could arguably have driven a common response in some proxies; (iii) this climate signal is likely to be, at least partly, a temperature indicator, because the proxy records were screened so that only those that were positively correlated with their local instrumental temperatures were selected (table S1); (iv) the analysis of instrumental temperatures indicates that 14 good temperature proxies are sufficient to represent NH mean temperature on 20-year and longer time scales; and (v) the comparison of results using proxy records and instrumental temperatures confirms that the analysis of these proxy records is a useful indicator of NH temperatures.

On this basis it is reasonable to conclude that this study provides evidence for intervals of significant warmth in the NH within the so-called Medieval Warm Period and for significantly colder intervals during the so-called Little Ice Age period. The most widespread and thus strongest evidence indicative of a significantly warm period occurs during the twentieth century [see also Supporting Online Material (SOM) Text], when greenhouse gas concentrations were at their highest during the analysis period. The proxy records indicate that the most widespread warmth occurred in either the mid- or late-twentieth century, but instrumental temperatures provide unequivocal evidence for continuing geographic expansion of anomalous warmth through to the present time.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/841/DC1
Materials and Methods
SOM Text
Figs. S1 to S6
Tables S1 and S2

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Histone H4-K16 Acetylation Controls Chromatin Structure and Protein Interactions

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Acetylation of histone H4 on lysine 16 (H4-K16Ac) is a prevalent and reversible posttranslational chromatin modification in eukaryotes. To characterize the structural and functional role of this mark, we used a native chemical ligation strategy to generate histone H4 that was homogeneously acetylated at K16. The incorporation of this modified histone into nucleosomal arrays inhibits the formation of compact 30-nanometer–like fibers and impedes the ability of chromatin to form cross-fiber interactions. H4-K16Ac also inhibits the ability of the adenosine triphosphate–utilizing chromatin assembly and remodeling enzyme ACF to mobilize a mononucleosome, indicating that this single histone modification modulates both higher order chromatin structure and functional interactions between a nonhistone protein and the chromatin fiber.

DNA in eukaryotes is present as chromatin, which is an assembly of histones, DNA, and chromatin-associated proteins. The basic building block of chromatin is the nucleosome, which contains two copies of histones H2A, H2B, H3, and H4 (*1*). Fifteen to 38 amino acids from each histone N terminus form the histone "tails," providing a platform for posttranslational modifications that modulate the biological role played by the underlying DNA (*2*). One prevalent modification is H4-K16Ac (*3*), which has roles in transcriptional activation and the maintenance of euchromatin (*4*, *5*).

Recent work has focused on the ability of histone marks to modulate the binding of nonhistone proteins to the chromatin fiber, such as the yeast silencing factor Sir3 and the *Drosophila* chromatin-remodeling enzyme ISWI (*6*, *7*). We were interested in testing whether histone modifications might control higher order chromatin structures. Indeed, random hyperacetylation of histone tails (>6 acetates per octamer) disrupts intramolecular folding of nucleosomal arrays into compact, 30-nm-thick fibers (*8*). Additionally, the H4 tail, and particularly residues 14 to 23, are uniquely important for the formation of these fibers (*1*, *9*). The acetylation of H4-K16 occurs within this region, providing a potential mechanism to regulate chromatin folding.

We used a native chemical ligation strategy to generate recombinant histone H4 homogeneously acetylated at K16 (*10*, *11*). In this strategy, an H4 N-terminal peptide (amino acids 1 to 22), with a C-terminal thioester and an acetylated lysine 16, was synthesized. A recombinant

C-terminal fragment of histone H4 (amino acids 23 to 102), in which H4 arginine 23 (R23) had been changed to a cysteine (R23C), was expressed and purified (fig. S1). Chemical ligation of these components yielded full-length H4-K16Ac (Fig. 1A). This product is the same length as unacetylated histone H4 (Fig. 1B) but demonstrates an expected reduction in charge (fig. S2). Three additional H4 polypeptides were expressed and purified (fig. S1): (i) wild-type (WT) H4; (ii) H4-R23C, which harbors the same cysteine substitution present in ligated H4; and (iii) H4-ΔN, in which the N-terminal tail (residues 1 to 19) has been deleted. All H4 polypeptides were incorporated into histone octamers containing recombinant H2A, H2B, and H3 (*1*), and neither H4-K16Ac nor ligation interfered with octamer assembly (Fig. 1B).

Using step-wise salt dialysis, the four distinct histone octamers were assembled into nucleosomal arrays with a DNA template that harbors 12 copies of the 177–base pair "601" nucleosome positioning sequence (601-177-12) (*9*, *12*). In order to ensure that each DNA template was saturated with 12 nucleosomes, octamers were added in slight excess to the number of 601 repeats, and mononucleosomal-length DNA was included in the reconstitution reactions to act as an "octamer sink" (Fig. 1C) (*9*). After assembly, 601-177-12 nucleosomal arrays were purified from mononucleosomes by selective $MgCl_2$ precipitation (Fig. 1C), and array saturation was confirmed (fig. S2).

We used sedimentation velocity in conjunction with van Holde–Weischet analysis (*13*) to ascertain the distribution of sedimentation coefficients for the population of nucleosomal arrays within a sample. Saturated arrays containing H4-WT (Fig. 2A, diamonds), H4-R23C (circles), H4-K16Ac (squares), and H4-ΔN octamers (triangles) were sedimented in a buffer lacking divalent cations, conditions in which nucleosomal arrays adopt an extended "beads-on-a-string" conformation. Each of the four arrays showed nearly identical distributions of sedimentation coefficients (S), with expected midpoints between 34 and 36 S (Fig. 2A, open symbols) (*9*, *14*).

When wild-type (Fig. 2E, solid diamonds) and H4-R23C (solid circles) arrays were incubated in a buffer containing 1.0 mM $MgCl_2$, they formed more compact fibers that shifted the sedimentation coefficient distributions to midpoints between 53 and 54 S. These results are consistent with the formation of compact, 30-nm–like fibers, and the H4-R23C substitution does not disrupt this condensation reaction. In contrast, and consistent with a previous report (*9*), the array reconstituted with H4 that lacks an N-terminal tail (H4-ΔN) (Fig. 2C, solid squares) was unable to condense fully, reaching a midpoint of only 44S. The array reconstituted with H4-K16Ac displayed an identical defect in $MgCl_2$-dependent compaction (Fig. 2A, solid triangles), suggesting that the acetylation of a single lysine leads to a chromatin folding defect equivalent to deletion of the entire H4 tail.

We also analyzed a large set (>10) of different subsaturated arrays (<12 nucleosomes

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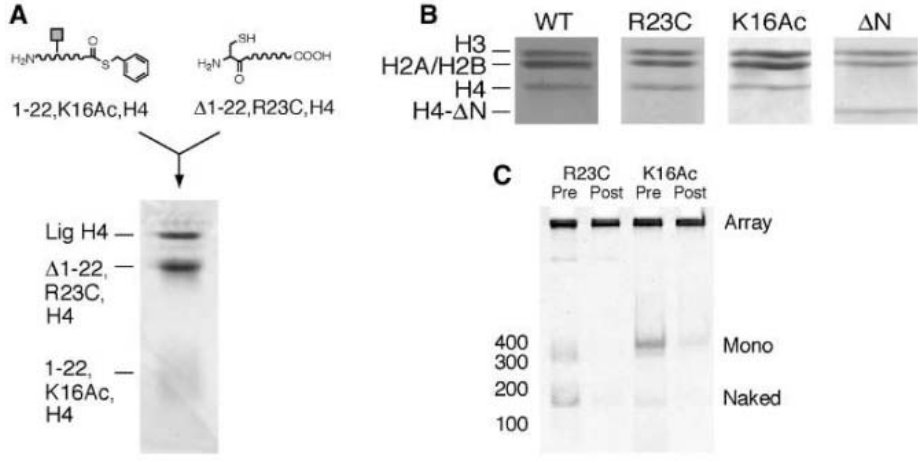

Fig. 1. Histone H4-K16Ac is incorporated into nucleosomal arrays. (**A**) Ligation of K16Ac-containing peptide and H4 C-terminal fragment. A ligation reaction is separated on 18% SDS-PAGE gel and stained with Coomassie (*27*). (**B**) Shown are peak fractions of H4-WT–, H4-R23C– H4-K16Ac–, or H4-ΔN–containing histone octamers isolated by gel filtration, separated on 18% SDS-PAGE gel, and stained with Coomassie. (**C**) Nucleosomal arrays containing histone H4-K16Ac prepared in the presence of 174–base pair (bp) 5S DNA. Nucleosomal arrays before (pre) and after (post) precipitation with 4.0 mM $MgCl_2$ analyzed on native 4% PAGE and stained with ethidium bromide are shown. Shown is a typical gel obtained with saturated arrays containing histone H4-R23C or histone H4-K16Ac. Mono and Naked indicate 5S mononucleosomes and free DNA, respectively.

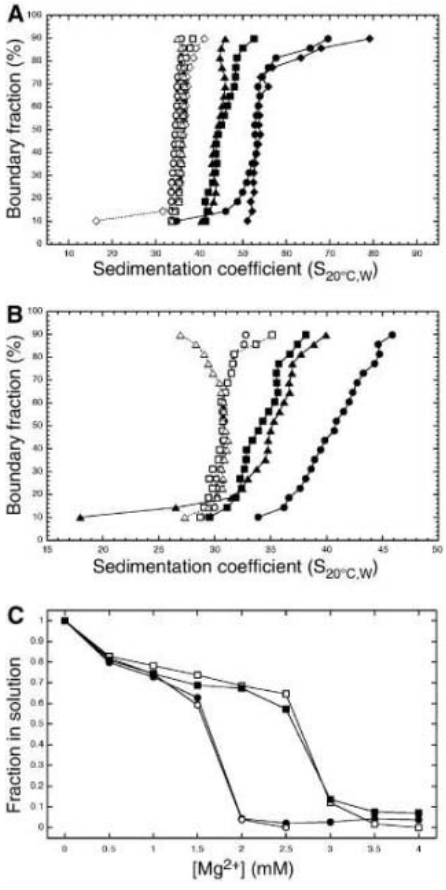

Fig. 2. H4-K16Ac abolishes higher order chromatin structure. (**A**) H4-K16Ac abolishes folding of nucleosomal arrays. Integrated sedimentation coefficient distributions of nucleosomal arrays in the presence and absence of Mg^{2+} were determined by using sedimentation velocity and van Holde-Weischet analysis. Arrays containing WT, H4-R23C, H4-K16Ac, and H4-ΔN histones are depicted by diamonds, circles, squares, and triangles, respectively. Arrays analyzed in the absence (0.1mM ethylenediaminetetraacetic acid) or presence of Mg^{2+} (1.0 mM $MgCl_2$) are shown as open or solid symbols, respectively. $S_{20°C,W}$ is the sedimentation coefficient corrected to water at 20°C and adjusted for the difference in mass of the H4-ΔN histone. The data shown are representative of 3 to 5 array reconstitutions. (**B**) Subsaturated arrays containing H4-K16Ac. Analysis was performed as in (**A**). Data shown are representative of at least 3 to 5 array reconstitutions. (**C**) H4-K16Ac disrupts array oligomerization. Nucleosomal arrays were incubated with varying concentrations of $MgCl_2$ at room temperature for 15 min, followed by centrifugation in a microfuge. The fraction of array remaining in the supernatant is plotted as a function of $MgCl_2$ concentration. Arrays containing WT, H4-R23C, H4-K16Ac, and H4-ΔN histones are depicted by solid circles, open circles, open squares, and solid squares, respectively.

per template) reconstituted with the four different octamers (Fig. 2B) (*15*). In every case, the addition of $MgCl_2$ to the wild-type and H4-R23C arrays led to large increases in the sedimentation coefficient distributions, consistent with salt-dependent compaction. However, arrays reconstituted with the H4-ΔN and H4-K16Ac octamers were again equally defective for $MgCl_2$-dependent compaction at every level of saturation (Fig. 2B) (*15*).

As the concentration of $MgCl_2$ was increased beyond 1.5 mM, arrays underwent reversible self-association that is believed to mimic fiber-fiber interactions that stabilize higher order chromosomal domains (*16*). For the 601-177-12 arrays, the deletion of the H4 tail disrupted self-association (*9*). To test whether H4-K16Ac affects this intermolecular interaction, arrays reconstituted with H4-WT, H4-R23C, H4-K16Ac, and H4-ΔN octamers were assayed. Self-association of the H4-WT and H4-R23C arrays (Fig. 2C, solid and open circles, respectively) occurred at magnesium concentrations of 1.5 to 2.0 mM, comparable to previously reported values (*9*). In contrast, self-association of the H4-ΔN and H4-K16Ac arrays (Fig. 2C, solid and open squares, respectively) both required higher concentrations of $MgCl_2$ (2.5 to 3.0 mM). Thus, as in the case for intramolecular folding, the single acetylation of H4-K16 cripples the self-association of arrays and shows defects equivalent to the loss of the H4 tail.

Next we investigated whether H4-K16Ac is associated with decondensed chromatin structures in vivo. HeLa nuclei were digested with micrococcal nuclease (Mnase), and the released chromatin fractionated into $MgCl_2$-soluble and $MgCl_2$-insoluble components. Samples were separated on an SDS–polyacrylamide gel and on an acid-urea-triton (AUT) gel, and the abundance of H4-K16Ac was analyzed by Western blotting (Fig. 3). Consistent with the biochemical studies, H4-K16Ac was enriched in the $MgCl_2$-soluble chromatin fractions (Fig. 3, A and B) that are also enriched in transcriptionally active gene sequences (*17*). Furthermore, the $MgCl_2$-soluble fractions contained an H4 tail that is exclusively monoacetylated at H4-K16 (Fig. 3B).

We also tested whether nucleosomal H4-K16Ac affects interactions with chromatin-associated proteins, specifically the *Drosophila* ISWI-containing adenosine triphosphate (ATP)–utilizing chromatin assembly and remodeling enzyme (ACF) complex. This complex hydrolyzes ATP to mediate nucleosome sliding in cis along DNA (*18*). This activity requires residues 16 to 19 of the H4 tail (*19*, *20*), and in vitro peptide competition assays have suggested that H4-K16Ac may reduce the interaction of ISWI with nucleosomes (*7*). To test directly the effect of H4-K16Ac on ISWI activity, we reconstituted end-positioned mononucleosomes with wild-type, H4-R23C, and H4-K16Ac octamers. Each of these mononucleosomes was incubated with the ACF complex, and ATP-dependent mobilization of nucleosomes was analyzed by native polyacrylamide gel electrophoresis (PAGE). When wild-type nucleosomes were incubated with ACF and ATP, a slower-migrating species accumulated with time (half-time $t_{1/2}$ = ~7.5 min) (Fig. 4.). This shift in electrophoretic mobility depended on the presence of both ACF and ATP, and it likely represents sliding of the nucleosome to a more central position. This ACF activity was also detected on mononucleosomes reconstituted with H4-K16Ac, but in this case, sliding was slower as compared to the WT and H4-R23C substrates ($t_{1/2}$ = ~20 min). These results are consistent with previous peptide studies, demonstrating that H4-K16Ac regulates the functioning of a chromatin-remodeling enzyme independent of its effects on chromatin higher order structure.

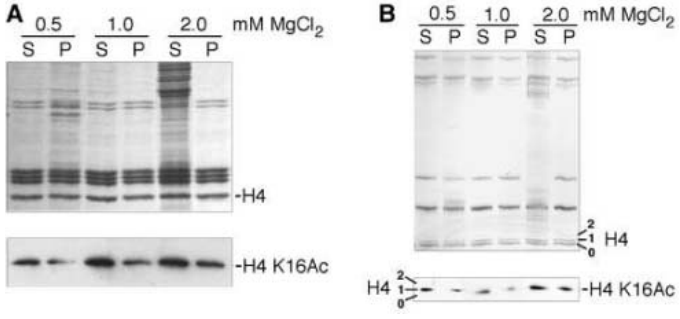

Fig. 3. H4-K16Ac is enriched in $MgCl_2$-soluble chromatin. HeLa nuclei were digested with micrococcal nuclease, and the solubilized chromatin was incubated with $MgCl_2$ at different final concentrations of 0.5, 1, or 2 mM for 10 min on ice. Histones from the magnesium soluble (S) and insoluble (P) chromatin fractions were electrophoresed on (**A**) 15% SDS-PAGE gels or (**B**) 15% AUT-PAGE gels. The AUT gel separates the acetylated histone isoforms. The top panels display Coomassie blue–stained gels and the bottom panels show Western analyses with antibodies to H4-K16Ac (Upstate, Charlottesville, VA).

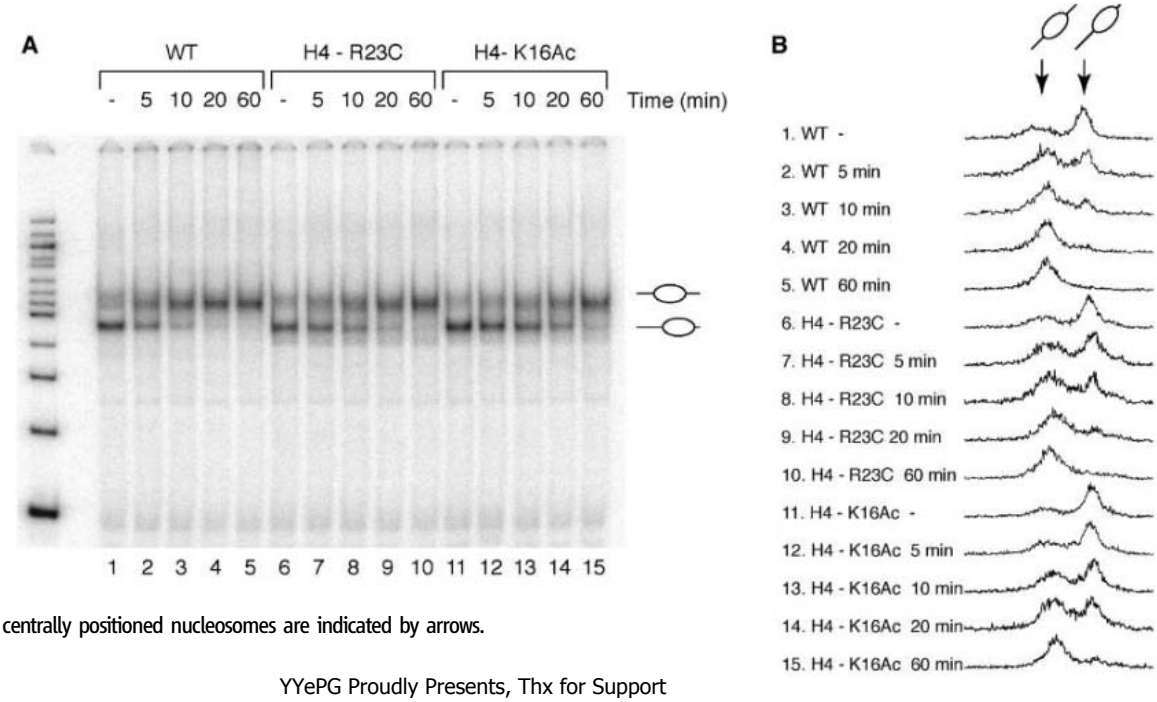

Fig. 4. H4-K16Ac inhibits ACF-mediated nucleosome sliding. (**A**) End-positioned nucleosomes containing indicated histone H4 were incubated with ACF and ATP at 30°C. Samples were taken from reactions at indicated times and quenched by the addition of EDTA and by placing them on ice. Samples were separated by electrophoresis on 4% native polyacrylamide gels. Lanes 1, 6, and 11 show mononucleosomes without incubation with ACF. (**B**) Profiles of gel lanes shown in (A). Peaks corresponding to the predicted end-positioned and centrally positioned nucleosomes are indicated by arrows.

The structural effect of H4-K16Ac may directly contribute to regions of decondensed chromatin in eukaryotic organisms. In budding yeast, over 80% of H4 is acetylated at lysine 16, and most of the genome exists in a decondensed state (*3*, *21*). Likewise, evidence suggests that the transcriptionally enhanced X chromosome of male flies, a site of ubiquitous H4-K16Ac, is decondensed (*22*). Such decondensation of chromatin may contribute to the establishment of transcriptionally active euchromatic regions. In vitro transcription studies suggest that the adoption of higher order chromatin structure reduces gene transcription (*23*). In contrast, acetylation of H4-K16 increases gene transcription both in vitro and in vivo (*24*), and the decompaction resulting from such modification may increase the accessibility of factors that promote transcription.

Do other histone marks regulate chromatin folding? The phosphorylation of H3-S10 (S, serine) does not disrupt chromatin folding (*25*), and triacetylation of the H3 tail by Gcn5p does not disrupt chromatin compaction (*26*). Similarly, residues 1 to 13 of histone H4 that include three sites of acetylation are dispensable for folding of 601-177-12 arrays (*9*). Thus, H4-K16 is likely to be a unique acetylation site of histone tails, which function as a dual switch for higher order chromatin structure and protein-histone interactions, promoting chromatin function in a mutually reinforcing manner.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/844/DC1
Materials and Methods
Figs. S1 to S3
References

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Caspases 3 and 7: Key Mediators of Mitochondrial Events of Apoptosis

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The current model of apoptosis holds that upstream signals lead to activation of downstream effector caspases. We generated mice deficient in the two effectors, caspase 3 and caspase 7, which died immediately after birth with defects in cardiac development. Fibroblasts lacking both enzymes were highly resistant to both mitochondrial and death receptor–mediated apoptosis, displayed preservation of mitochondrial membrane potential, and had defective nuclear translocation of apoptosis-inducing factor (AIF). Furthermore, the early apoptotic events of Bax translocation and cytochrome c release were also delayed. We conclude that caspases 3 and 7 are critical mediators of mitochondrial events of apoptosis.

Mitochondria play a central role in apoptosis. Mitochondrial outer membrane permeabilization (MOMP) leads to release of proapoptotic factors such as cytochrome c and AIF (*1*). Furthermore, loss of mitochondrial membrane potential ($\Delta\psi_m$) is thought to contribute to cell death by disruption of normal mitochondrial function (*2*, *3*). Interaction of members of the Bcl-2 family of proteins regulates MOMP, the key event of cytochrome c release into the cytoplasm (*3*, *4*). What is less clear, however, is the precise role of caspase proteases in mitochondrial events of apoptosis. Although upstream caspases, such as caspase 2 and caspase 8, affect mitochondrial events in both death-receptor and mitochondrial pathways of apoptosis, either directly or through interaction with Bcl-2 family members, the role of presumed downstream "effector" caspases in this process is less clear (*5*, *6*). Therefore, we studied the two highly related effectors, caspase 3 and caspase 7, to elucidate their functions in apoptosis.

We generated caspase $7^{-/-}$ mice (fig. S1), which were born in ratios consistent with Mendelian inheritance. They had normal appearance, organ morphology, and lymphoid development. When caspase $7^{-/-}$ mouse embryonic fibroblasts (MEFs) were treated with inducers of apoptosis, they exhibited a slight survival advantage as compared with wild-type MEFs. Apoptosis caused by a range of insults in other caspase $7^{-/-}$ cells proceeded normally, however, including the death of activated T cells following stimulation of the T cell receptor, thymocyte apoptosis, Fas-mediated death of B cells, and Fas-mediated death of hepatocytes (fig. S2).

Caspase 3, which is structurally similar to caspase 7, might compensate for the lack of caspase 7, which would lead to this relatively mild antiapoptotic phenotype (*7*, *8*). Thus, we bred caspase $7^{-/-}$ mice to caspase $3^{-/-}$ mice previously described by our laboratory (*9*). The embryonic stem cells containing the mutation were from the 129/SvJ genetic background. Mice derived from these embryonic stem cells were backcrossed six generations onto the C57BL/6 background. We obtained no live caspase $3^{-/-}$/caspase $7^{-/-}$ double-knockout (DKO) mice when progeny were genotyped at an age of 10 to 14 days. DKO mice were present at normal Mendelian numbers through embryonic day 20 (E20), but died rapidly after birth. A small percentage (~10%) of both caspase $3^{-/-}$/caspase $7^{+/-}$ and DKO embryos displayed exencephaly, likely due to the absence of caspase 3 in combination with residual genes from the 129/SvJ background (*10*). The major-

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ity of E20 DKO embryos had a grossly normal appearance. This suggests that neither caspase 3 nor caspase 7 are important for brain development on the C57BL/6 genetic background.

Histologic examination of DKO hearts revealed dilation of the atria (Fig. 1, A and B) and disorganization and noncompaction of the ventricular musculature (Fig. 1, C through F). This noncompaction is similar to that of mice deficient in the death receptor–signaling molecules caspase 8, FADD, and c-FLIP (caspase 8–related protein) (*11*–*13*), although all of these mice exhibit additional developmental abnormalities and die in midgestation. Thus, caspases 3 and 7 together are important for proper cardiac development, and noncompaction may occur because they act downstream of death receptor signaling. Other aspects of death receptor–mediated development, however, leading to earlier lethality in caspase 8, FADD, and c-FLIP knockout mice, likely proceed through alternative pathways.

To examine the combined functions of caspases 3 and 7 in apoptosis, we tested the sensitivity of MEFs to two inducers of mitochondrially mediated apoptosis—ultraviolet (UV) irradiation and staurosporine, and two activators of the death receptor pathway—Fas ligand (FasL) and tumor necrosis factor–α (TNFα). DAPI (4′,6-diamidinole-2-phenolindole) staining of the nuclei of UV irradiated cells revealed typical morphologic characteristics of cell death in caspase $3^{+/-}$/caspase $7^{+/-}$ (Fig. 2A) and caspase $3^{+/-}$/caspase $7^{-/-}$ (Fig. 2B) cells. Caspase $3^{-/-}$/caspase $7^{+/-}$ MEFs displayed distorted chromatin condensation (Fig. 2C), and nuclear fragmentation was absent (*14*). In contrast, UV-irradiated DKO MEFs maintained a normal nuclear morphology (Fig. 2D), even 24 hours after treatment (Fig. 2, E and F). At 24 hours after irradiation, caspase $3^{+/-}$/caspase $7^{+/-}$ (Fig. 2G) and caspase $3^{+/-}$/caspase $7^{-/-}$ (Fig. 2H) MEFs underwent complete loss of cellular morphology, and many cells detached from the plate. Some caspase $3^{-/-}$/caspase $7^{+/-}$ MEFs partially retained gross morphology (Fig. 2I), but their cytoplasm was contracted. DKO MEFs, on the other hand, appeared completely normal (Fig. 2J), with flattened, attached cell bodies.

Consistent with this, DKO MEFs displayed increased viability after stimulation of the death-receptor pathway (Fig. 2K) as compared with that of other genotypes. Caspase $3^{-/-}$/caspase $7^{+/-}$ and caspase $3^{+/-}$/caspase $7^{-/-}$ cells showed an intermediate effect. Interestingly, caspase $7^{-/-}$ MEFs showed a consistently greater viability than caspase $3^{-/-}$ MEFs. In contrast, when we measured DNA fragmentation by nucleosome enzyme-linked immunosorbent assay (ELISA) (Fig. 2L) caspase $3^{-/-}$ MEFs showed complete absence of DNA fragmentation, whereas both caspase $7^{-/-}$ and caspase $3^{+/-}$/caspase $7^{-/-}$ cells showed DNA fragmentation comparable to that of the wild type. Furthermore, cleavage of the caspase substrate poly(adenosine diphosphate–ribose) polymerase was entirely dependent on caspase 3 and not caspase 7 (Fig. 2M). These results show that caspases 3 and 7 have some overlapping, but also some distinct, roles in apoptosis. Caspase 3 controls DNA fragmentation and morphologic changes of apoptosis, whereas caspase 7 plays little role in these processes. In contrast, caspase 7 appears to be more important to the loss of cellular viability, although the combined role of both caspases is crucial in this area.

We transferred fetal liver cells into RAG-deficient mice and saw a normal distribution of $CD4^+$, $CD8^+$, and double-positive thymocytes in the DKO (fig. S3). We subjected these thymocytes to apoptotic stimuli. Determination of the fraction of cells with hypodiploid ($subG_0$) DNA content (Fig. 3A), reflecting the nuclear changes seen with apoptosis, showed a complete resistance in DKO and a slightly smaller degree of resistance in caspase $3^{-/-}$/caspase $7^{+/-}$ thymocytes. When analyzed for viability (Fig. 3B), however, only DKO thymocytes were resistant to apoptosis mediated by the mitochondrial pathway, although they were susceptible to death receptor–mediated apoptosis. These data imply that caspases 3 and 7 are not necessary for positive and negative selection of thymocytes. They further support a primary role for caspase 3 in DNA fragmentation, and a combined role of caspases 3 and 7 in viability. Because DKO thymocytes die normally from death-receptor stimuli, some cells, such as thymocytes, may have alternative pathways to loss of viability that bypass caspases 3 and 7.

The central role of mitochondria in cell death is highlighted by the importance of $\Delta\psi_m$. This gradient is critical for normal mitochondrial function, and its loss is associated with apoptosis (*3*). Inhibition of caspase activity protects dying cells from loss of $\Delta\psi_m$, at least in part through cleavage of respiratory chain proteins, but the identity of the caspase(s) involved has been unclear (*15*–*17*). We examined $\Delta\psi_m$ after treatment of MEFs with UV irradiation (Fig. 4A). In DKO MEFs, $\Delta\psi_m$ was preserved, whereas the other genotypes showed a decrease in the magnitude of $\Delta\psi_m$. These results identify caspases 3 and 7 as key mediators of the loss of $\Delta\psi_m$ that occurs during apoptosis.

Mitochondria in cells undergoing stress-induced apoptosis may act as amplifiers of caspase activity, much as they do in death receptor–mediated apoptosis (*18*, *19*). We investigated whether caspases may act as amplifiers of mitochondrial events. In DKO cells, early mitochondrial events—Bax translocation to the mitochondria and cytochrome c release

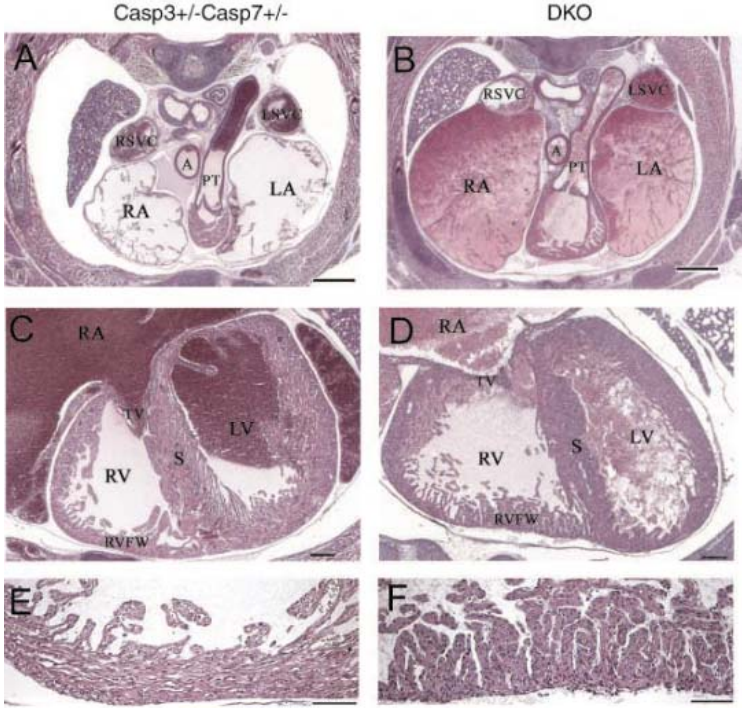

Fig. 1. (**A** to **F**) Defective cardiac development in DKO embryos. Hematoxylin-and-eosin staining of transverse sections of caspase $3^{+/-}$/caspase $7^{+/-}$ and DKO E20 embryo hearts. Higher magnification of the right ventricular free wall (E and F). Scale bars: (A and B), 500 μm; (C and D), 200 μm; and (E and F), 100 μm. Abbreviations: A, aorta; PT, pulmonary trunk; RA, right atrium; LA, left atrium; RSVC, right superior vena cava; LSVC, left superior vena cava; LV, left ventricle; RV, right ventricle; S, septum; TV, tricuspid valve; RVFW, right ventricular free wall.

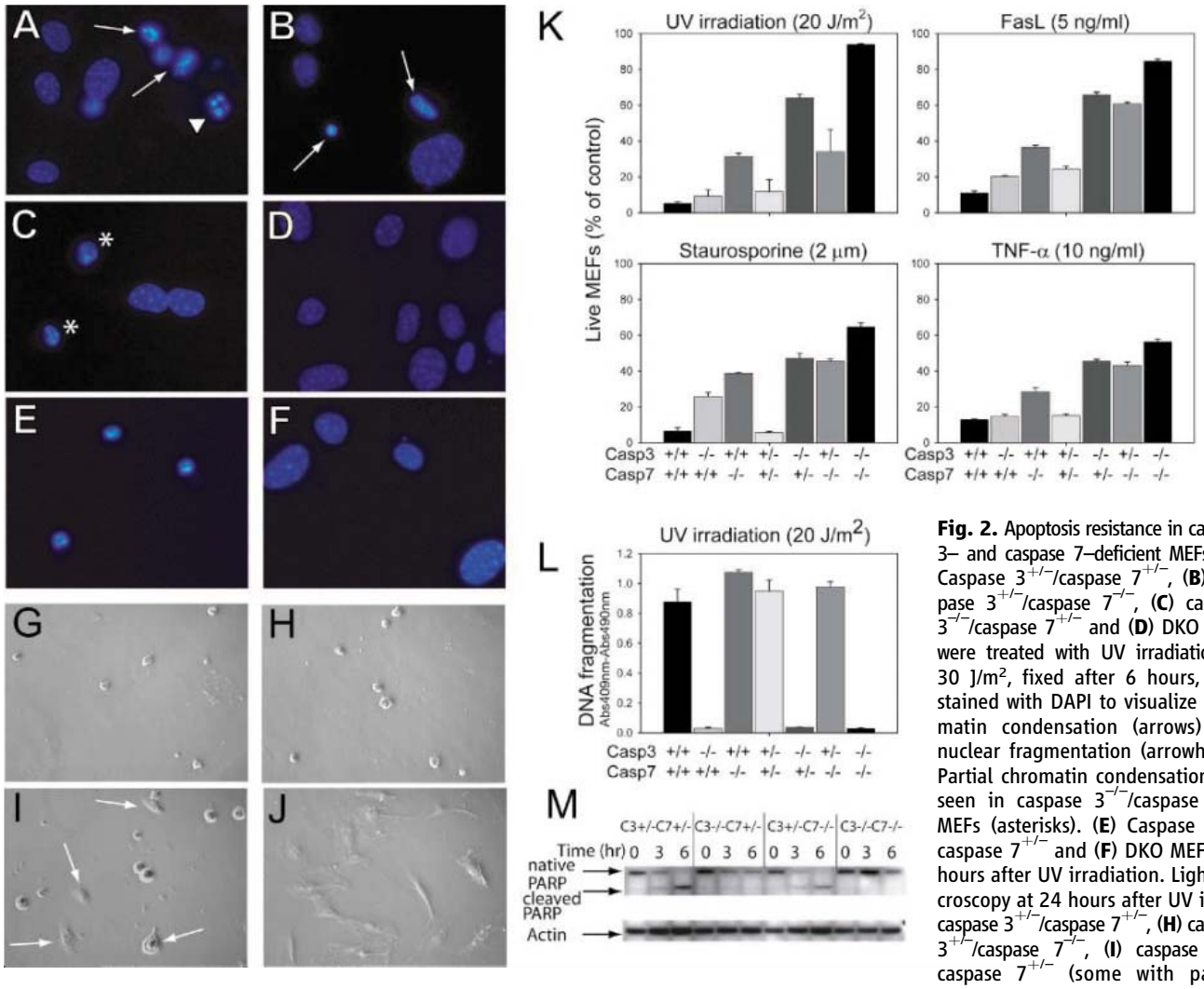

Fig. 2. Apoptosis resistance in caspase 3– and caspase 7–deficient MEFs. (**A**) Caspase $3^{+/-}$/caspase $7^{+/-}$, (**B**) caspase $3^{+/-}$/caspase $7^{-/-}$, (**C**) caspase $3^{-/-}$/caspase $7^{+/-}$ and (**D**) DKO MEFs were treated with UV irradiation at 30 J/m^2, fixed after 6 hours, then stained with DAPI to visualize chromatin condensation (arrows) and nuclear fragmentation (arrowhead). Partial chromatin condensation was seen in caspase $3^{-/-}$/caspase $7^{+/-}$ MEFs (asterisks). (**E**) Caspase $3^{+/-}$/caspase $7^{+/-}$ and (**F**) DKO MEFs, 24 hours after UV irradiation. Light microscopy at 24 hours after UV in (**G**) caspase $3^{+/-}$/caspase $7^{+/-}$, (**H**) caspase $3^{+/-}$/caspase $7^{-/-}$, (**I**) caspase $3^{-/-}$/caspase $7^{+/-}$ (some with partial preservation of morphology, arrows), and (**J**) DKO MEFs. (**K**) Improved survival in DKO MEFs. MEFs were treated as indicated, FasL and TNF-α each supplemented with cyclohexamide (10 μg/ml). After 24 hours, survival was determined using a live/dead cytotoxicity/viability assay. Data are means ± SD from a single experiment in triplicate, representative of at least three separate experiments. $P < 0.01$ for pairwise comparison of differences between DKO and each of the other six genotypes. (**L**) DNA cleavage is associated with caspase 3 alone. Cells were treated with UV and harvested after 3 hours, then nucleosome ELISA was done to measure DNA fragmentation. Data are means ± SD from a single experiment in duplicate, representative of at least three separate experiments. (**M**) PARP cleavage is a function of caspase 3. Cells were treated with UV (20 J/m^2) and harvested at indicated time points, then Western blots were performed on whole-cell lysates.

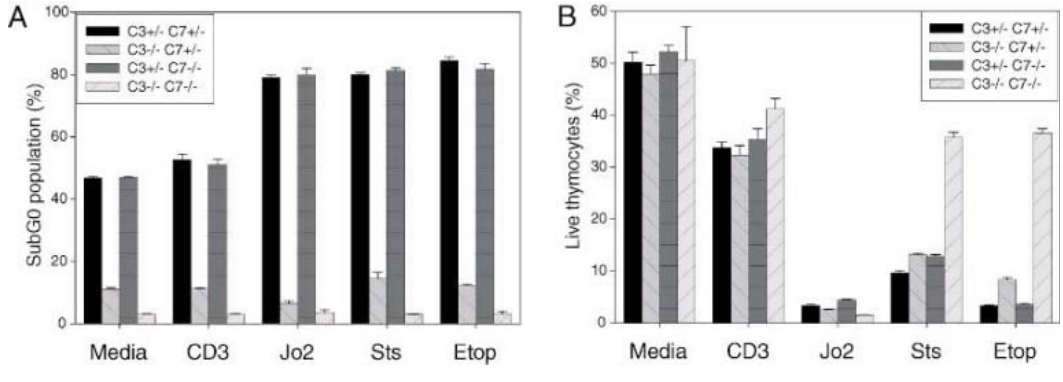

Fig. 3. Thymocyte apoptosis. (**A**) Reduced subG$_0$ population in caspase $3^{-/-}$/caspase $7^{+/-}$ and DKO thymocytes. Thymocytes from RAG$^{-/-}$ chimeras were plated in media alone or treated with plate-bound antibodies against CD3 and CD28 (20 μg/ml each), the Fas-specific antibody Jo2 (1 μg/ml) + cyclohexamide (10 μg/ml), staurosporine (0.1 μM), or etoposide (25 μM). Cells were fixed at 24 hours and stained with propidium iodide. SubG$_0$ population was identified by using flow cytometry in the FL-2 channel and was expressed as a percentage of total cells. Data are means ± SD from a single experiment in triplicate, representative of at least three separate experiments. For each treatment, $P < 0.001$ for the difference between DKO and C3KO. (**B**) Reduced mitochondrially mediated cell death in DKO thymocytes. Thymocytes with above treatments were analyzed at 24 hours using a live-dead cytotoxicity-viability assay, and viable cells were expressed as a percentage of total cells analyzed. Data are means ± SD from a single experiment in triplicate, representative of at least three separate experiments.

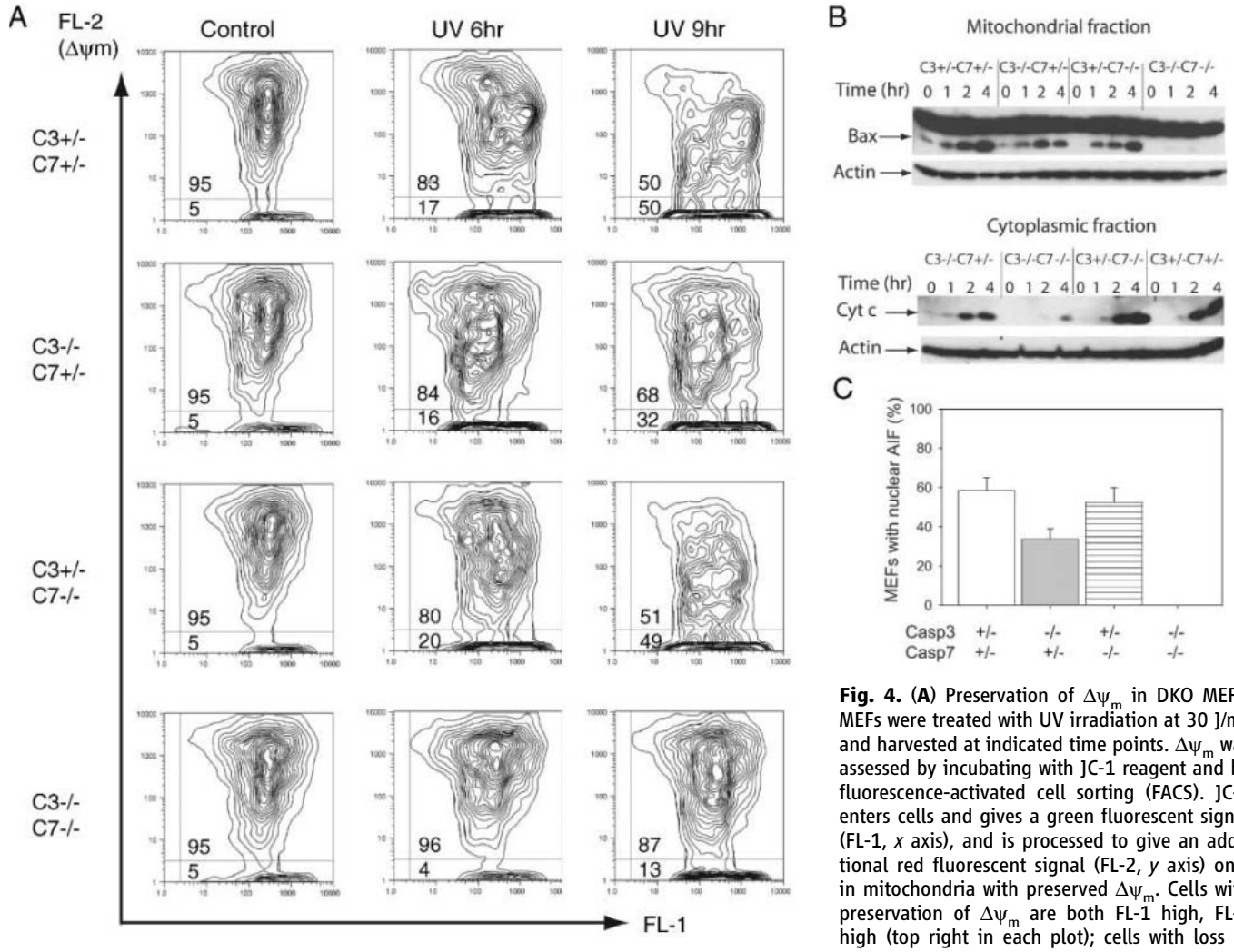

Fig. 4. (**A**) Preservation of $\Delta\psi_m$ in DKO MEFs. MEFs were treated with UV irradiation at 30 J/m^2 and harvested at indicated time points. $\Delta\psi_m$ was assessed by incubating with JC-1 reagent and by fluorescence-activated cell sorting (FACS). JC-1 enters cells and gives a green fluorescent signal (FL-1, *x* axis), and is processed to give an additional red fluorescent signal (FL-2, *y* axis) only in mitochondria with preserved $\Delta\psi_m$. Cells with preservation of $\Delta\psi_m$ are both FL-1 high, FL-2 high (top right in each plot); cells with loss of $\Delta\psi_m$ are FL-1 high, FL-2 low (bottom right in each plot). FACS data plots are shown, with percentage of total events indicated. Data are from a single experiment, representative of at least three independent experiments. (**B** and **C**) Control of release of mitochondrial apoptotic factors by caspases 3 and 7. (B) Delayed Bax translocation and cytochrome c release in DKO MEFs. MEFs treated with UV irradiation at 30 J/m^2, mitochondrial (top) and cytoplasmic (bottom) fractions were separated at indicated time points. Western blots were probed with indicated antibodies. (C) Absent AIF nuclear translocation in DKO MEFs. MEFs were treated with UV irradiation at 30 J/m^2 and fixed after 6 hours. Cells with AIF nuclear translocation were counted in five random fields and expressed as a percentage of total cells (minimum 120 total cells counted per genotype). Data are means ± SD from a single experiment, representative of two separate experiments.

into the cytoplasm—were both delayed (Fig. 4B). Similar findings were also made at early time points for caspase $9^{-/-}$ MEFs (fig. S4). Notably, caspase 9 processing was normal in DKO MEFs, whereas caspases 2 and 8 were not processed in either wild-type or DKO MEFs (fig. S4). This suggests a pathway from caspase 9 through caspases 3 and 7 that promotes cytochrome c release at early time points. These events did occur ultimately, as living, healthy-appearing DKO MEFs displayed Bax translocation and cytochrome c release at later time points (fig. S5).

Translocation of the mitochondrial flavoprotein AIF to the nucleus is associated with chromatin condensation and DNA cleavage during apoptosis (*20*). Data using chemical inhibitors of caspase activity or cells deficient in single caspases, have suggested that AIF release may be either caspase-dependent or caspase-independent (*21*). Given the profound absence of chromatin condensation in DKO cells, we examined AIF release with immunocytochemistry (fig. S6). DKO MEFs completely lacked AIF translocation (Fig. 4C), whereas caspase $3^{-/-}$/caspase $7^{+/-}$ MEFs had a partial deficiency. These data are consistent with a role for AIF in chromatin condensation and DNA cleavage and indicate that, under these conditions, AIF release is fully dependent on the combined action of caspases 3 and 7.

The data presented here show that caspases 3 and 7 are crucial for apoptosis and contribute to some mitochondrial events once thought to lie upstream of effector caspases. They control the loss of $\Delta\psi_m$ and AIF release. They may also serve to amplify the initial death signal by helping to promote further cytochrome c release. Nevertheless, as Bax translocation and cytochrome c release occur eventually in DKO cells, there are likely multiple routes to regulate mitochondrial function in apoptosis.

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Translational Regulators Maintain Totipotency in the *Caenorhabditis elegans* Germline

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The molecular mechanisms that maintain totipotency of the germline are not well understood. Here, we show that two conserved translational regulators, MEX-3 and GLD-1, are essential for maintaining totipotency in the *Caenorhabditis elegans* germline. In *mex-3 gld-1* mutants, germ cells transdifferentiate into various somatic cell types such as muscles or neurons. Our findings implicate RNA regulation in the maintenance of totipotency, suggest that multiple mechanisms maintain totipotency at different stages of germline development, and establish a genetically tractable model for studying the development of teratomas.

How cells maintain or lose totipotency is a major question in stem cell and germ cell research (*1*). Germ cell precursors in early *C. elegans* and *Drosophila melanogaster* embryos maintain totipotency in part by transiently inhibiting transcription (*2*). Germ cells in larval and adult gonads are transcriptionally active and presumably require different mechanisms to maintain totipotency. The *C. elegans* hermaphrodite gonad contains germ cells in a linear sequence of developmental stages: proliferating germ cells in the distal gonad, meiotic cells in the central gonad, and cells undergoing spermatogenesis (late larvae) or oogenesis (adults) in the proximal gonad (Fig. 1A) (*2*). Many events in germline development, such as the mitosis/meiosis and spermatogenesis/oogenesis switches, involve translational regulation by the GLD-1 protein (*3*–*7*). GLD-1 is expressed primarily in the central gonad (*8*) and is a member of the signal transduction and activation of RNA (STAR) family of KH-domain, RNA binding proteins that includes mammalian Quaking and Sam68 (*9*). MEX-3 is expressed in a complementary pattern (Fig. 1A) (*10*–*12*); MEX-3 is the founding member of a distinct family of proteins with two KH-domains but is otherwise dissimilar from GLD-1. Whereas MEX-3 appears to function as a translational regulator (*10*, *13*), the functions of human orthologs such as Tino [with 83% and 75% identity to the first and second KH domains of MEX-3 (*14*)] remain unknown. Recent studies have shown that animals lacking GLD-1 misexpress MEX-3 in meiotic germ cells (*10*, *12*), raising the possibility that ectopic MEX-3 activity may contribute to previously characterized *gld-1*(–) phenotypes.

We constructed and examined *mex-3(or20) gld-1(q485)* double mutants (hereafter called *mex-3 gld-1*) lacking MEX-3 and GLD-1 activities. Many nuclei in the central gonad of the double mutants did not resemble germ nuclei in the light microscope but instead resembled nuclei found in somatic tissues (Fig. 1B). Similar nuclei were observed at a low frequency in the gonads of *gld-1* adults but were not present in *mex-3* mutants ($n > 100$) (fig. S1). Using

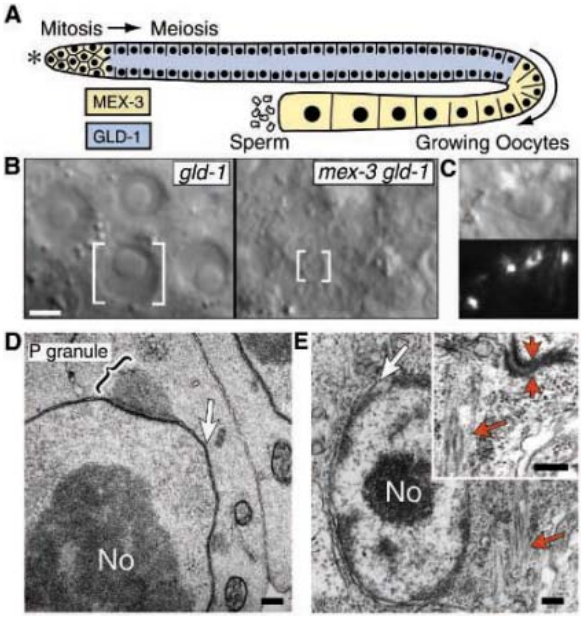

Fig. 1. Ectopic somatic cells in *mex-3 gld-1* gonads. (**A**) Diagram of wild-type gonad showing expression of MEX-3 (yellow) and GLD-1 (blue). The central region consists largely of germ nuclei at the pachytene stage of meiosis; an asterisk indicates the distal, mitotic zone. (**B**) Light micrographs of germ cells in 1-day-old *gld-1* or *mex-3 gld-1* adults; one nucleus in each gonad is bracketed. The *gld-1* germ cell nucleus resembles a wild-type germ cell nucleus (not shown) with a large nucleolus and clear nucleoplasm. *mex-3 gld-1* gonads contain some small nuclei (right) with granular nucleoplasm typical of heterochromatin in differentiated somatic cells. (**C**) The panels show light (top) and fluorescence (bottom) micrographs of a cell in a *mex-3 gld-1* gonad with birefringent-autofluorescent "gut granules." (**D**) Electron micrograph of a wild-type germ cell indicating the nucleolus (No), nuclear envelope (arrow), and a P granule (bracket). (**E**) Cell in a *mex-3 gld-1* gonad with a small nucleus, with prominent heterochromatin associated with the nuclear envelope (white arrow), and lacking P granules. The red arrow points to apparent myofilaments (magnified in the inset). An apparent adhesive junction typical of muscle cells is visible in the inset (short arrows). Scale bars: (B) 3 μm; [(D) and (E)] 0.2 μm. See (*31*).

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light and electron microscopy, immunostaining, and transgenic reporters, we confirmed that the abnormal cells were differentiated somatic cells, including two types of muscle (body and pharyngeal), neurons, and intestinal cells (Fig. 1, C and E; Fig. 2, A and B; and fig. S2, A and B). The muscles contained filaments and adhesive structures resembling those found in normal muscles (Fig. 1E), expressed muscle-specific markers (Fig. 2A and fig. S2, A and B), and contracted. The neurons expressed a neuronal-specific green fluorescent protein (GFP) reporter and had extensive processes similar to normal neurons (Fig. 2, A and B, and fig. S2, A and B). Finally, some *mex-3 gld-1* gonads (35 of 134) contained cells with birefringent and autofluorescent granules characteristic of wild-type intestinal cells (Fig. 1C) (*15*). Ectopic "somatic" cells were present in *gld-1*, but not *mex-3*, single mutants at a lower frequency (1 of 143 gonads of *gld-1* mutants contained gut granules) (Fig. 2, A and C). Germ cells in wild-type gonads contain ribonucleoprotein structures called P granules that are absent from somatic cells (*2*); similar structures are uniquely associated with germ cells in a wide range of animals (*16*). The ectopic somatic cells in *mex-3 gld-1* gonads appeared to lack P granules (compare Fig. 1, D and E) and did not express the P-granule proteins PGL-1, GLH-1, and GLH-4 (*17*, *18*).

Several lines of evidence suggest that the ectopic somatic cells are transdifferentiated germ cells. The gonad primordium in a newly hatched larva contains two germ cell precursors that generate the entire germline and two cells that produce all of the other gonadal cells. When the two germ cell precursors were killed with a laser in *mex-3 gld-1* larvae, the adult gonads did not contain ectopic somatic cells (0 of 12 operated gonads and 30 of 30 control gonads contained cells with neuronal-specific GFP). Thus, the presence of germ cells is required for the ectopic somatic cells. Additional experiments showed that the ectopic somatic cells did not result from inappropriate fertilization events (table S1) or from the spontaneous activation of unfertilized oocytes. *mex-3 gld-1* gonads did not contain cells resembling mature oocytes (fig. S1), nor did they accumulate at least some oocyte-specific proteins such as OMA-1/MOE-1 (*19*). Moreover, old, unfertilized wild-type oocytes that activate spontaneously and undergo numerous rounds of DNA replication did not express muscle myosin or neuronal GFP (0 of 38 endoreplicated oocytes from 2- to 2.5-day-old wild-type adults). We found that germ cells in the central region of young adult *mex-3 gld-1* gonads (42 of 43 gonads) showed a marked reduction in the size and numbers of P granules before the appearance of ectopic somatic cells (Fig. 3A and table S1). We thus consider it likely that these aberrant germ cells are the precursors of the ectopic somatic cells that later appear in the central gonad and that lack P granules.

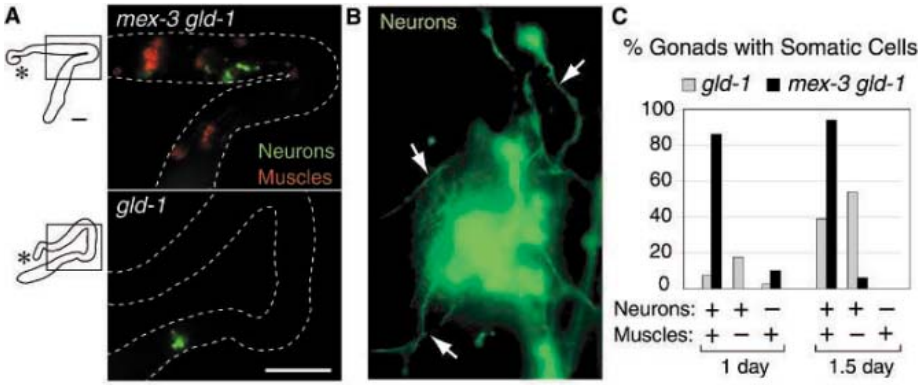

Fig. 2. MEX-3 and GLD-1 prevent transdifferentiation of germ cells. (**A**) Micrographs of 1-day-old *gld-1* or *mex-3 gld-1* adult gonads (outlined) showing clusters of apparent neurons (green) or muscles (red). Images are of boxed regions shown in the gonad diagrams at left; asterisks indicate distal tips. (**B**) High magnification of a neuronal cluster showing processes (arrows). Muscles were stained with monoclonal antibody (5.6) to myosin, and neurons expressed an *unc-119::GFP* transgene. (**C**) Quantitation of somatic differentiation in staged adult gonads (day 1: n = 40 *gld-1*, n = 29 *mex-3 gld-1*; day 1.5: n = 28 *gld-1*, n = 32 *mex-3 gld-1*).

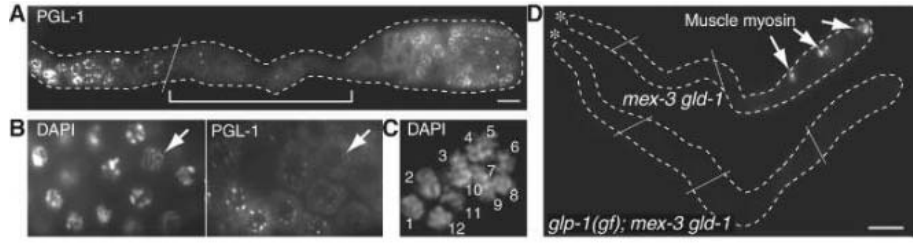

Fig. 3. P-granule loss and requirement for meiosis in transdifferentiating gonads. (**A**) A 0.5-day-old adult *mex-3 gld-1* gonad immunostained for the P-granule component PGL-1. P granules are apparent in the distal (left) and proximal (right) mitotic zones of the gonad but are diminished in the central zone (bracket). At this stage, P-granule defects were apparent in 42 of 43 gonads, although only 21 of 46 expressed the muscle factor HLH-1. (**B**) High magnification of *mex-3 gld-1* germ nuclei stained for DNA (left) and PGL-1 (right); the arrow indicates an apparent pachytene-stage nucleus lacking P granules. (**C**) High magnification of a single *mex-3 gld-1* germ nucleus with 12 chromosomes. (**D**) Gonads from a 1.5-day-old *mex-3(RNAi)gld-1(RNAi)* adult and a *glp-1(gf);mex-3(RNAi)gld-1(RNAi)* adult stained for muscle myosin. Clusters of muscles (arrows) were present in *mex-3(RNAi) gld-1(RNAi)* gonads (19 of 25 gonads) but not in *glp-1(gf);mex-3(RNAi)gld-1(RNAi)* gonads (day 1.5: n = 0 of 19; day 2: n = 0 of 57). Thin lines through gonads in this and other figures indicate boundaries of photographs used for composite images. Scale bars: (A) 20 μm; (D) 50 μm.

Somatic differentiation in *mex-3 gld-1* gonads is reminiscent of human germ cell tumors called teratomas, which contain somatic tissues such as neurons, teeth, or hair. Teratomas in male and female germlines are thought to result from distinct defects (*20*). Ovarian teratomas are the most common ovarian neoplasms and originate from germ cells that have entered, but not properly completed, meiosis (*21*, *22*). We found that the "worm teratoma" occurred only in germlines that initiated a female program of development (table S1). *C. elegans* female and male germ cells are different as early as in mitosis (*8*), but germ cell abnormalities are not apparent in *mex-3 gld-1* gonads until meiosis (Fig. 3B). Mutant germ cells at pachytene were often interspersed with aberrant germ cells containing up to 12 chromosomes (Fig. 3C). Because wild-type chromosomes pair to form six bivalents and remain paired until fertilization, the 12 chromosomes likely represent unpaired homologous chromosomes. These abnormalities suggest that defects in meiosis could contribute to transdifferentiation, although none of the meiotic mutants in *C. elegans* have been reported to undergo transdifferentiation. To address whether entry into meiosis was required for transdifferentiation, we used a *glp-1(oz112 gf)* gain-of-function mutation that forces germ cells to remain in mitosis (*23*). None of the *glp-1(gf);mex-3(RNAi)gld-1(RNAi)*

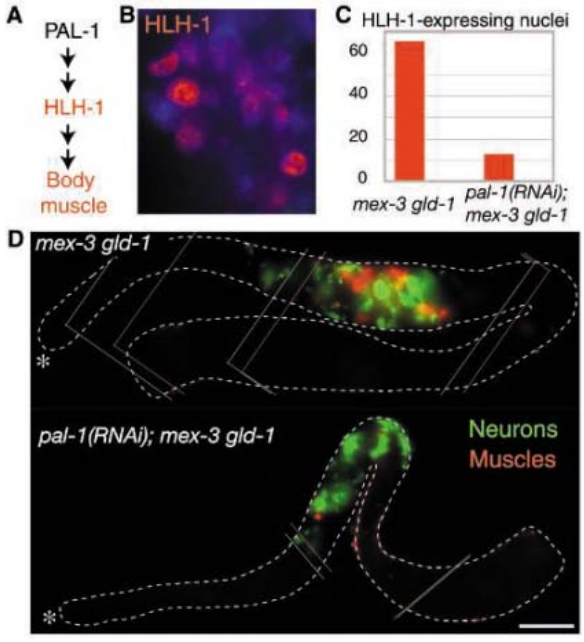

Fig. 4. PAL-1 is required for most body muscle transdifferentiation in *mex-3 gld-1* gonads. (**A**) Body muscle differentiation in normal embryogenesis involves PAL-1/Caudal and multiple downstream targets such as HLH-1/MyoD. (**B**) Inappropriate HLH-1 expression (red) in *mex-3 gld-1* germ nuclei; 4′,6′-diamidino-2-phenylindole staining shown in blue. (**C**) Quantitation of HLH-1–expressing nuclei per gonad arm; data from 12 *mex-3 gld-1* and 10 *pal-1(RNAi);mex-3 gld-1* gonads. Wild-type gonads show no detectable HLH-1 ($n > 50$). (**D**) Examples of mock-depleted (top) and PAL-1–depleted (bottom) *mex-3 gld-1* gonads. The remaining muscles in *pal-1(RNAi);mex-3 gld-1* gonads may be PAL-1–independent muscles (*24*) or may result from incomplete *pal-1(RNAi)*. Scale bar, 50 μm.

gonads contained somatic cells (Fig. 3D), suggesting that entry into meiosis is critical for transdifferentiation.

Little is known about the molecular pathways that induce teratomas (*20*). For our analysis of the *C. elegans* gonad, we focused on muscle differentiation. Most muscle precursors in normal embryogenesis are specified, in part, through a pathway that involves the transcriptional regulator PAL-1/Caudal and downstream factors such as HLH-1/MyoD (Fig. 4A) (*24*, *25*). HLH-1 was not detectable in wild-type germ nuclei but was present in large numbers of *mex-3 gld-1* germ nuclei (Fig. 4, B and C). Depletion of PAL-1 from *mex-3 gld-1* gonads caused a marked reduction in both the number of HLH-1–positive nuclei (Fig. 4C) and the number of body muscles (Fig. 4D). Thus, most of the ectopic body muscles appear to differentiate through a pathway that mimics a major muscle pathway in normal embryogenesis. Because PAL-1 and other factors that induce somatic differentiation in *C. elegans* embryos are encoded by maternally expressed mRNAs, this may explain why transdifferentiation does not occur in masculinized germlines (table S1).

Both MEX-3 and GLD-1 contribute to translational repression of *pal-1* mRNA in wild-type gonads (*11*–*13*). However, wild-type meiotic germ cells occasionally express PAL-1 without transdifferentiating (*12*). Thus, we consider it unlikely that inappropriate expression of PAL-1 is, by itself, sufficient to induce transdifferentiation. Moreover, *pal-1(RNAi);mex-3 gld-1* gonads that contain only a few body muscles contain numerous neurons and pharyngeal cells (Fig. 4D), suggesting the involvement of additional, PAL-1 independent, pathways of somatic differentiation. We propose that transdifferentiation involves both (i) the expression in germ cells of factors such as PAL-1 that normally regulate somatic differentiation in embryos and (ii) a defect that allows germ cells to respond to these factors. GLD-1 is required for wild-type meiotic germ cells to progress beyond the transcriptionally active pachytene stage of meiosis to diakinesis (*6*), where chromosomes are transcriptionally quiescent. A prolonged, aberrant pachytene stage might make germ cells sensitive to factors such as PAL-1. MEX-3 and GLD-1 regulate diverse mRNAs, and future studies should show whether specific target mRNAs have roles in transdifferentiation. For example, MEX-3/GLD-1–dependent regulation of chromatin modifiers might function in distinguishing germline and somatic states. One known GLD-1 target encodes a component of the histone H3 methyltransferase (*26*, *27*), and previous studies have shown that LET-418/Mi-2, a component of a *C. elegans* nucleosome-remodeling and histone deacetylase complex, prevents expression of germline proteins in somatic cells (*28*). The P-granule defects in *mex-3 gld-1* mutants might also contribute to transdifferentiation. P granules normally contain multiple maternally produced mRNAs and some regulators of RNA metabolism (*2*). In yeast and mammalian somatic cells, cytoplasmic structures with some similarity to P granules have a role in mRNA silencing and decay (*29*, *30*). Although no *C. elegans* mutant has been described that completely lacks P granules, P-granule defects might lead to the release and inappropriate expression of component mRNAs, resulting in transdifferentiation.

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31. Materials and methods are available as supporting material on *Science* Online.

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Supporting Online Material

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Figs. S1 and S2
Table S1
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Experimental Study of Inequality and Unpredictability in an Artificial Cultural Market

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Hit songs, books, and movies are many times more successful than average, suggesting that "the best" alternatives are qualitatively different from "the rest"; yet experts routinely fail to predict which products will succeed. We investigated this paradox experimentally, by creating an artificial "music market" in which 14,341 participants downloaded previously unknown songs either with or without knowledge of previous participants' choices. Increasing the strength of social influence increased both inequality and unpredictability of success. Success was also only partly determined by quality: The best songs rarely did poorly, and the worst rarely did well, but any other result was possible.

How can success in cultural markets be at once strikingly distinct from average performance (*1*–*4*), and yet so hard to anticipate for profit-motivated experts armed with extensive market research (*4*–*8*)? One explanation (*9*) for the observed inequality of outcomes is that the mapping from "quality" to success is convex (i.e., differences in quality correspond to larger differences in success), leading to what has been called the "superstar" effect (*9*), or "winner-take-all" markets (*10*). Because models of this type, however, assume that the mapping from quality to success is deterministic and that quality is known, they cannot account for the observed unpredictability of outcomes. An alternate explanation that accounts for both inequality and unpredictability asserts that individuals do not make decisions independently, but rather are influenced by the behavior of others (*11*, *12*). Stochastic models of collective decisions that incorporate social influence can exhibit extreme variation both within and across realizations (*4*, *13*, *14*), even for objects of identical quality (*3*, *15*). Unfortunately, empirical tests of these predictions require comparisons between multiple realizations of a stochastic process, whereas in reality, only one such "history" is ever observed.

We adopted an experimental approach to the study of social influence in cultural markets. We created an artificial "music market" (*16*) comprising 14,341 participants, recruited mostly from a teen-interest World Wide Web site (*17*), who were shown a list of previously unknown songs from unknown bands (*18*). In real time, arriving participants were randomly assigned to one of two experimental conditions—independent and social influence—distinguished only by the availability of information on the previous choices of others. In the independent condition, participants made decisions about which songs to listen to, given only the names of the bands and their songs. While listening to a song, they were asked to assign a rating from one star ("I hate it") to five stars ("I love it"), after which they were given the opportunity (but not required) to download the song. In the social influence condition, participants could also see how many times each song had been downloaded by previous participants. Thus, in addition to their own musical preferences, participants in the social influence condition received a relatively weak signal regarding the preferences of others, which they were free to use or ignore. Furthermore, participants in the social influence condition were randomly assigned to one of eight "worlds," each of which evolved independently of the others. Songs in each world accumulated downloads only from participants in that world, and subsequent participants could only see their own world's download counts.

Our experimental design has three advantages over both theoretical models and observational studies. (i) The popularity of a song in the independent condition (measured by market share or market rank) provides a natural measure of the song's quality, capturing both its innate characteristics and the existing preferences of the participant population. (ii) By comparing outcomes in the independent and social influence conditions, we can directly observe the effects of social influence both at the individual and collective level. (iii) We can explicitly create multiple, parallel histories, each of which can evolve independently. By studying a range of possible outcomes rather than just one, we can measure inherent unpredictability: the extent to which two worlds with identical songs, identical initial conditions, and indistinguishable populations generate different outcomes. In the presence of inherent unpredictability, no measure of quality can precisely predict success in any particular realization of the process.

We report the results of two experiments in which we study the outcomes for 48 songs by different bands (*18*). In both experiments, all songs started with zero downloads (i.e., all initial conditions were identical), but the presentation of the songs differed. In the social influence condition in experiment 1, the songs, along with the number of previous downloads, were presented to the participants arranged in a 16×3 rectangular grid, where the positions of the songs were randomly assigned for each participant (i.e., songs were not ordered by download counts). Participants in the independent condition had the same presentation of songs, but without any information about previous downloads. In experiment 2, participants in the social influence condition were shown the songs, with download counts, presented in one column in descending order of current popularity. Songs in the independent condition were also presented with the single column format, but without download counts and in an order that was randomly assigned for each participant. Thus, in each experiment, we can observe the effect of social influence on each song's success, and by comparing results across the two experiments, we can measure the effect of increasing the "strength" of the relevant information signal.

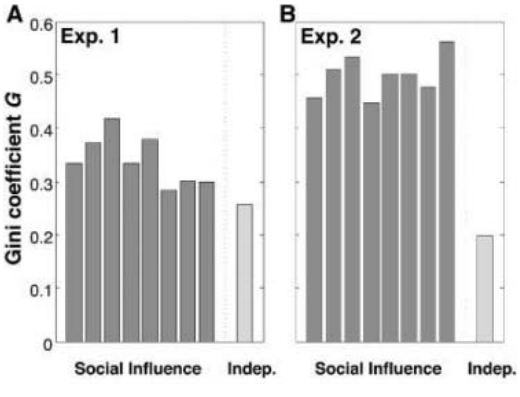

Fig. 1. Inequality of success for social influence (dark bars) and independent (light bars) worlds for (**A**) experiment 1 and (**B**) experiment 2. The success of a song is defined by m_i, its market share of downloads ($m_i = d_i / \sum_{k=1}^{S} d_k$, where d_i is song i's download count and S is the number of songs). Success inequality is defined by the Gini coefficient $G = \sum_{i=1}^{S} \sum_{j=1}^{S} |m_i - m_j| / 2S \sum_{k=1}^{S} m_k$, which represents the average difference in market share for two songs normalized to fall between 0 (complete equality) and 1 (maximum inequality). Differences between independent and social influence conditions are significant ($P < 0.01$) (*18*).

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Our results support the hypothesis that social influence, which here is restricted only to information regarding the choices of others, contributes both to inequality and unpredictability in cultural markets. Figure 1 displays the effects of social influence on market inequality, as measured by the Gini coefficient (*19*) (other measures yield similar results). In both experiments, we found that all eight social influence worlds (dark bars) exhibit greater inequality—meaning popular songs are more popular and unpopular songs are less popular—than the world in which individuals make decisions independently (light bars). Comparing Fig. 1, A and B, we also note that inequality increased when the salience of the social information signal was increased from experiment 1 to experiment 2. Thus our results suggest not only that social influence contributes to inequality of outcomes in cultural markets, but that as individuals are subject to stronger forms of social influence, the collective outcomes will become increasingly unequal.

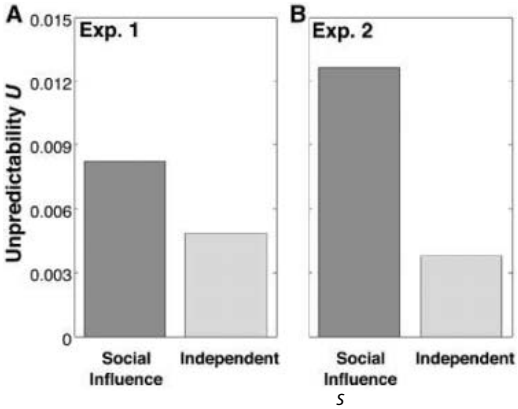

Fig. 2. Unpredictability of success for (**A**) experiment 1 and (**B**) experiment 2. In both experiments, success in the social influence condition was more unpredictable than in the independent condition. Moreover, the stronger social signal in experiment 2 leads to increased unpredictability. The measure of unpredictability u_i for a single song i is defined as the average difference in market share for that song between all pairs of realizations; i.e., $u_i = \sum_{j=1}^{W} \sum_{k=j+1}^{W} |m_{i,j} - m_{i,k}| / \binom{W}{2}$, where $m_{i,j}$ is song i's market share in world j and W is the number of worlds. The overall unpredictability measure $U = \sum_{i=1}^{S} u_i / S$ is then the average of this measure over all S songs. For the independent condition, we randomly split the single world into two subpopulations to obtain differences in market shares, and we then averaged the results over 1000 of these splits. All differences are significant ($P < 0.01$) (*18*).

Social influence also generates increased unpredictability of outcomes (Figs. 2 and 3). In each experiment, the average difference in market share (fraction of total downloads) for a song between distinct social influence worlds is higher than it is between different subpopulations of individuals making independent decisions (Fig. 2). Because these different outcomes occur even with indistinguishable groups of subjects evaluating the same set of songs, this type of unpredictability is inherent to the process and cannot be eliminated simply by knowing more about the songs or market participants. Figure 3 displays the market share (left column) and market rank (right column) of each song in each of the eight social influence worlds as a function of its "quality" (i.e., its market share and rank, respectively, in the independent condition). Although, on average, quality is positively related to success, songs of any given quality can experience a wide range of outcomes (Fig. 3). In general, the "best" songs never do very badly, and the "worst" songs never do extremely well, but almost any other result is possible. Unpredictability also varies with quality—measured in terms of market share, the "best" songs are the most unpredictable, whereas when measured in terms of rank, intermediate songs are the most unpredictable (this difference derives from the inequality in success noted above). Finally, a comparison of Fig. 3, A and C, suggests that the explanation of inequality as arising from a convex mapping between quality and success (*9*) is incomplete. At least some of the convexity derives not from similarity of preexisting preferences among market participants, but from the strength of social influence.

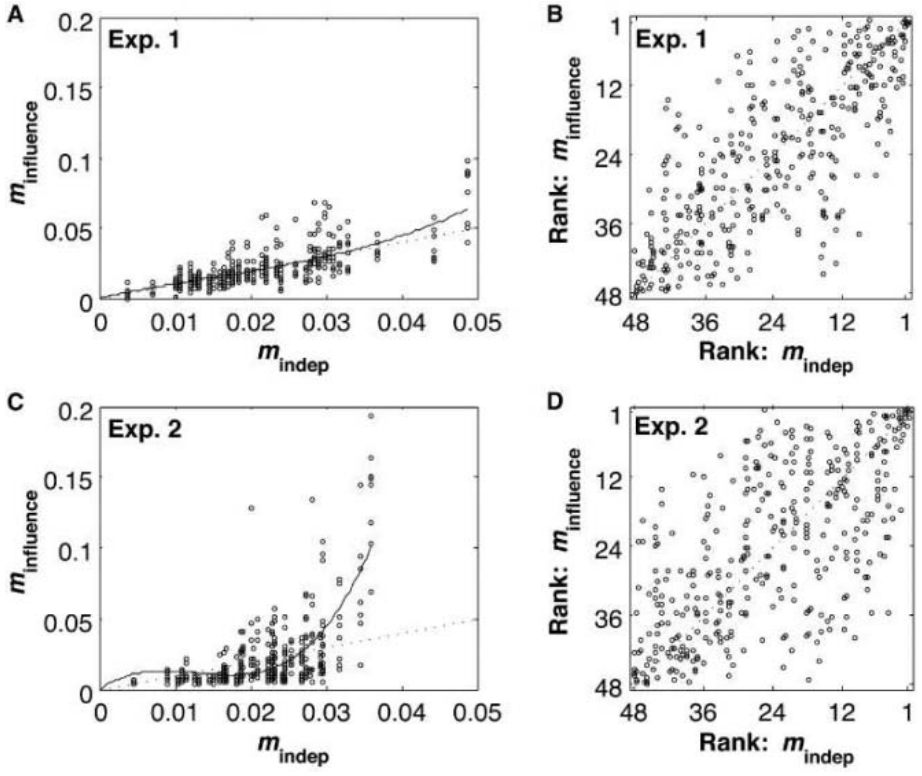

Fig. 3. Relationship between quality and success. (**A**) and (**C**) show the relationship between m_{indep}, the market share in the one independent world (i.e., quality), and $m_{influence}$, the market share in the eight social influence worlds (i.e., success). The dotted lines correspond to quality equaling success. The solid lines are third-degree polynomial fits to the data, which suggest that the relationship between quality and success has greater convexity in experiment 2 than in experiment 1. (**B**) and (**D**) present the corresponding market rank data.

Our experiment is clearly unlike real cultural markets in a number of respects. For example, we expect that social influence in the real world—where marketing, product placement, critical acclaim, and media attention all play important roles—is far stronger than in our experiment. We also suspect that the effects of social influence were further diminished by the relatively small number of songs, and by our requirements (which aided control) that subjects could participate only once and could not share opinions. Although these differences limit the immediate relevance of our experiment to real-world cultural markets, our findings nevertheless suggest that social influence exerts an important but counterintuitive effect on cultural market formation, generating collective behavior that is reminiscent of (but not identical to) "information cascades" in sequences of individuals making binary choices (*20*–*22*). On the one hand, the more information participants have regarding the decisions of others, the greater agreement

they will seem to display regarding their musical preferences; thus the characteristics of success will seem predictable in retrospect. On the other hand, looking across different realizations of the same process, we see that as social influence increases (i.e., from experiment 1 to experiment 2), which particular products turn out to be regarded as good or bad becomes increasingly unpredictable, whether unpredictability is measured directly (Fig. 2) or in terms of quality (Fig. 3). We conjecture, therefore, that experts fail to predict success not because they are incompetent judges or misinformed about the preferences of others, but because when individual decisions are subject to social influence, markets do not simply aggregate pre-existing individual preferences. In such a world, there are inherent limits on the predictability of outcomes, irrespective of how much skill or information one has.

Although Web-based experiments of the kind used here are more difficult to control in some respects than are experiments conducted in physical laboratories (*18*), they have an important methodological advantage for studying collective social processes like cultural market formation. Whereas experimental psychology, for example, tends to view the individual as the relevant unit of analysis, we are explicitly interested in the relationship between individual (micro) and collective (macro) behavior; thus we need many more participants. In order to ensure that our respective worlds had reached reasonably steady states, we required over 14,000 participants—a number that can be handled easily in a Web-based experiment, but which would be impractical to accommodate in a physical laboratory. Because this "micro-macro" feature of our experiment is central to all collective social dynamics (*23*), we anticipate that Web-based experiments will become increasingly useful to the study of social processes in general.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/854/DC1
Materials and Methods
SOM Text
Figs. S1 to S10
Tables S1 to S4
References

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The Nucleosomal Surface as a Docking Station for Kaposi's Sarcoma Herpesvirus LANA

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Kaposi's sarcoma–associated herpesvirus (KSHV) latency-associated nuclear antigen (LANA) mediates viral genome attachment to mitotic chromosomes. We find that N-terminal LANA docks onto chromosomes by binding nucleosomes through the folded region of histones H2A-H2B. The same LANA residues were required for both H2A-H2B binding and chromosome association. Further, LANA did not bind *Xenopus* sperm chromatin, which is deficient in H2A-H2B; chromatin binding was rescued after assembly of nucleosomes containing H2A-H2B. We also describe the 2.9-angstrom crystal structure of a nucleosome complexed with the first 23 LANA amino acids. The LANA peptide forms a hairpin that interacts exclusively with an acidic H2A-H2B region that is implicated in the formation of higher order chromatin structure. Our findings present a paradigm for how nucleosomes may serve as binding platforms for viral and cellular proteins and reveal a previously unknown mechanism for KSHV latency.

Kaposi's sarcoma–associated herpesvirus (KSHV) has an etiological role in Kaposi's sarcoma (KS), the predominant AIDS malignancy; primary effusion lymphoma (PEL); and multicentric Castleman's disease (*1*–*4*). KSHV persists as a multicopy episome in latently infected tumor cells (*5*, *6*). Viral genomes lack centromeres, which govern faithful DNA partitioning in eukaryotic cells, and use a distinct segregation mechanism in which the 1162–amino acid KSHV latency-associated nuclear antigen (LANA) tethers episomes to mitotic chromosomes. LANA is required for episome persistence, and interaction with mitotic chromosomes is essential for its function. The first 22 residues comprise the dominant LANA chromosome-association region, because the C-terminal chromosome targeting domain is unable to rescue chromosome association in mutants that are deleted for or contain specific mutations within the N-terminal region (*7*–*10*). We therefore sought to determine the chromosome docking partner of the LANA N terminus.

Genetic analysis of LANA's chromosome binding region was central to our strategy for characterization of putative docking partners. Transient assays have shown that alanine substitutions at LANA residues 5 to 7 [original amino acids were GMR (*11*)], 8 to 10 (originally LRS), or 11 to 13 (originally GRS) (termed LANA $_{5}$GMR$_{7}$, LANA $_{8}$LRS$_{10}$, and LANA $_{11}$GRS$_{13}$, respectively) (Fig. 1A) lack chromosome association, whereas LANA with alanine substitutions at amino acids 17 to 19 (originally PLT) or 20 to 22 (originally RGS) (termed LANA $_{17}$PLT$_{19}$ and LANA $_{20}$RGS$_{22}$,

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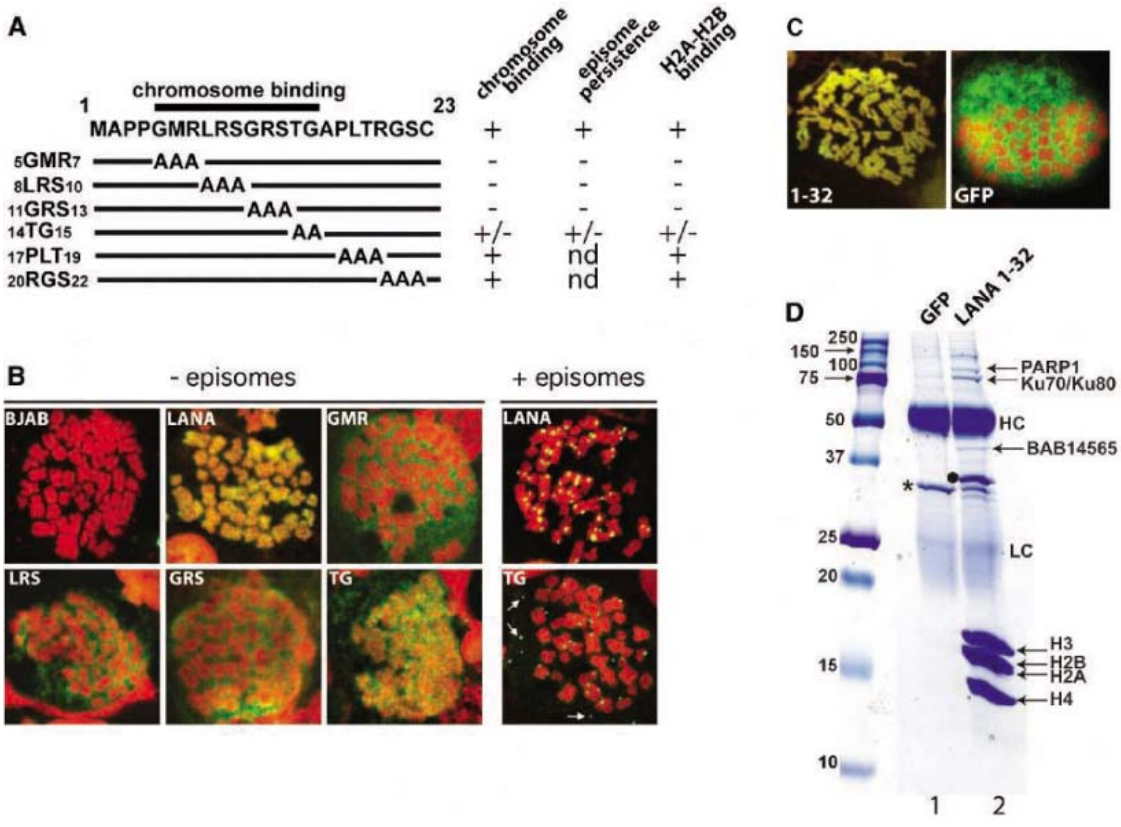

Fig. 1. LANA N terminus chromosome binding. (**A**) LANA scanning alanine mutants with summaries for chromosome binding, episome persistence (*7*), and H2A-H2B binding. nd, not determined. (**B**) Metaphase spreads of BJAB cells and BJAB cells stably expressing LANA, $LANA_5GMR_7$, $LANA_8LRS_{10}$, $LANA_{11}GRS_{13}$, or $LANA_{14}TG_{15}$. Overlay of LANA (green) and chromosomes (red) generates yellow. Cells containing KSHV episomes are indicated. Arrows denote $LANA_{14}TG_{15}$ dots that have detached from chromosomes. Magnification is 630×. (**C**) Metaphase BJAB cells stably expressing GFP NLS or GFP LANA 1-32 at 630× magnification. (**D**) Proteins co-precipitating with GFP LANA 1-32 (lane 2) were identified after resolution in a 4 to 16% gradient gel. HC, heavy chain; LC, light chain; asterisk, GFP; ●, GFP LANA 1-32. The stoichiometry of histones within nucleosomes and their arginine-rich nature contribute to the intense histone Coomassie staining. Numbers on the left-hand side of the gel are size markers (kD).

respectively) associates with chromosomes (Fig. 1A). LANA with alanine substitutions at residues $_{14}TG_{15}$ (termed LANA $_{14}TG_{15}$) may have reduced affinity for chromosomes (*7*). To further investigate LANA $_{14}TG_{15}$, we stably expressed these mutants in uninfected BJAB cells at amounts similar to those of LANA in infected PEL cells. LANA (green) tightly associated with chromosomes (red) (overlay generates yellow), whereas LANA $_5GMR_7$, LANA $_8LRS_{10}$, and LANA $_{11}GRS_{13}$ (green) did not (Fig. 1B). LANA $_{14}TG_{15}$ (green) associated with chromosomes (red) (overlay generates yellow) but also distributed between chromosomes, indicating weak association. We also investigated LANA $_{14}TG_{15}$ chromosome association in cells with KSHV episomes. In contrast to its broad distribution over chromosomes in the absence of KSHV episomes, LANA concentrates to dots along mitotic chromosomes at sites of episomes, consistent with its role in tethering KSHV DNA to chromosomes (*5*, *12*). Although LANA dots always tightly associated with chromosomes, ~30% of mitotic cells had LANA $_{14}TG_{15}$ dots that were detached from chromosomes (Fig. 1B, arrows). Because LANA dots are sites of KSHV DNA, $LANA_{14}TG_{15}$ dots not associated with chromosomes indicate inefficient episome partitioning. This finding follows our previous observation that LANA $_{14}TG_{15}$ is deficient in supporting episome persistence (*7*).

To identify N-terminal LANA's mitotic chromosome binding partner, we affinity-purified interacting proteins. BJAB cells stably expressing green fluorescent protein (GFP) fused to LANA residues 1 to 32 (GFP LANA 1-32), or GFP fused with a nuclear localization signal (GFP NLS), were generated (Fig. 1C). GFP does not affect LANA's chromosome localization (*7*) or negate its ability to mediate episome persistence (*13*). Proteins that interacted specifically with GFP LANA 1-32 were identified by co-immunoprecipitation followed by mass spectrometry (Fig. 1D). These included large amounts of core histones H2A, H2B, H3, and H4, as well as Ku70, Ku80, poly(adenosine diphosphate-ribose) polymerase 1 (PARP1), and BAB14565, a protein with high homology to the histone variant macroH2A. We determined with the use of knockout mouse embryo fibroblasts (MEFs) that Ku70, Ku80, and PARP1 do not mediate LANA chromosome association [fig. S1 and Supporting Online Material (SOM) Text].

The diffuse distribution of the LANA N terminus over mitotic chromosomes and the efficient precipitation of core histones strongly suggested that core histones mediate LANA chromosome docking. To further investigate this possibility, we assayed whether N-terminal LANA bound histones during mitosis. GFP LANA 1-32 was immunoprecipitated from asynchronous cells (~5% mitotic) (Fig. 2A, lane 2), or from metaphase-arrested cells (~85% mitotic) (Fig. 2A, lane 5). Despite the 17-fold difference in mitotic index, core histones precipitated similarly from asynchronous and metaphase-arrested cells. These results indicate that LANA associates with core histones throughout most or all of the cell cycle.

We determined whether full-length LANA also associated with core histones. GFP LANA 1-32 and GFP LANA, but not GFP NLS, efficiently precipitated core histones after expression in COS cells (Fig. 2B). We also investigated LANA's association with core histones in KSHV-infected BCBL-1 PEL cells. After incubation with a monoclonal antibody against LANA or with polyclonal serum, histone H2B was precipitated from BCBL-1 cells but not uninfected BJAB cells (Fig. 2C). Therefore, LANA interacts with core histones in KSHV-infected tumor cells.

We investigated whether the LANA N terminus directly binds nucleosome core particles (NCPs), which consist of two copies each of core histones H2A, H2B, H3, and H4, organizing ~147 base pairs (bp) of DNA (*14*). Glutathione *S*-transferase (GST) LANA 1-23, but not GST, directly bound and precipitated purified nucleosomes (Fig. 2D). Further, GST LANA 1-23 supershifted recombinant nucleosomes in a native gel (Fig. 2E). Because GST LANA 1-23 does not interact with purified DNA (*15*), binding was specific to the histone component of nucleosomes.

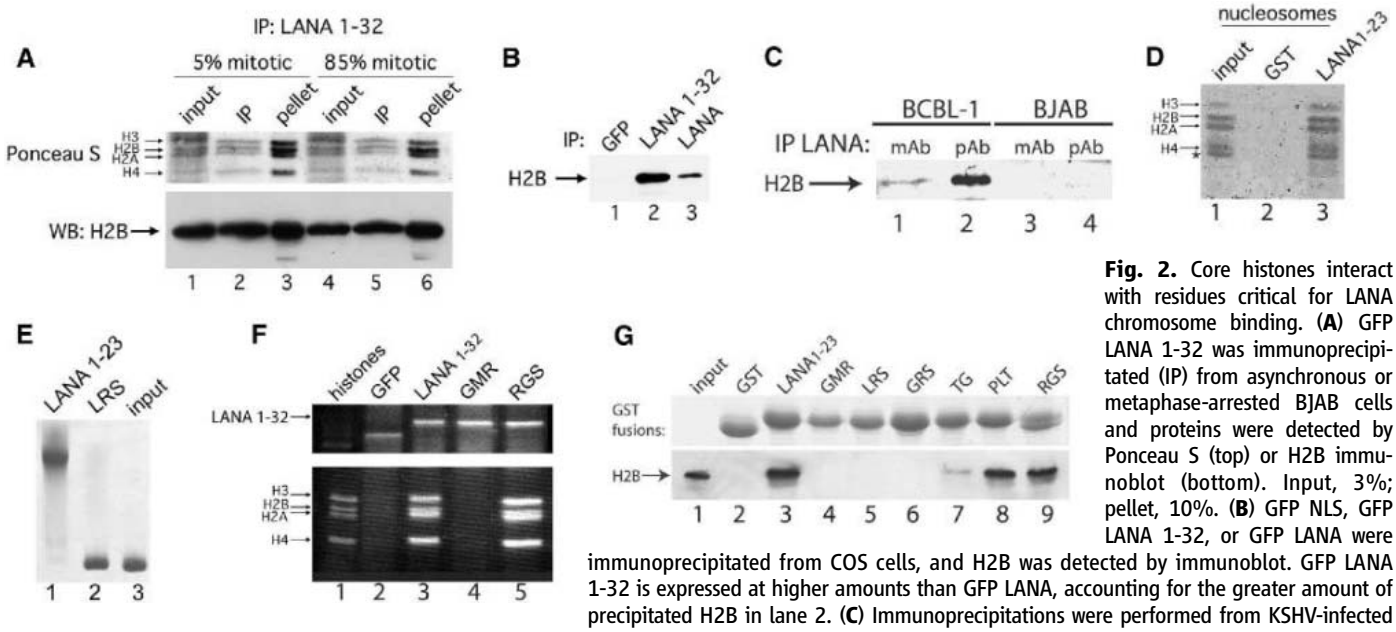

Fig. 2. Core histones interact with residues critical for LANA chromosome binding. (**A**) GFP LANA 1-32 was immunoprecipitated (IP) from asynchronous or metaphase-arrested BJAB cells and proteins were detected by Ponceau S (top) or H2B immunoblot (bottom). Input, 3%; pellet, 10%. (**B**) GFP NLS, GFP LANA 1-32, or GFP LANA were immunoprecipitated from COS cells, and H2B was detected by immunoblot. GFP LANA 1-32 is expressed at higher amounts than GFP LANA, accounting for the greater amount of precipitated H2B in lane 2. (**C**) Immunoprecipitations were performed from KSHV-infected BCBL-1 cells or uninfected BJAB cells by using monoclonal antibody (mAb) against LANA or polyclonal serum (pAb). H2B was detected by immunoblot. (**D**) H1-depleted nucleosomes were incubated with GST or GST LANA 1-23, and precipitated histones were detected by Coomassie. Input, 30%. Asterisk, degradation product. (**E**) Nucleosomes were incubated with GST LANA 1-23 or GST LANA 1-23 $_{8}$LRS$_{10}$, resolved by 5% native polyacrylamide gel electrophoresis, and detected by Coomassie. (**F**) Proteins immunoprecipitated by GFP or GFP fusions were detected by SYPRO Ruby (Invitrogen). Lane 1, purified histones. (**G**) GST fusion proteins were incubated with H1-depleted nucleosomes. GST fusions were detected by Coomassie, and precipitated H2B was detected by immunoblot.

We next investigated whether core histones interact with LANA residues necessary for chromosome association. GFP LANA 1-32 and GFP LANA 1-32 $_{20}$RGS$_{22}$, which associate with chromosomes, precipitated core histones from COS cells, whereas GFP LANA 1-32 $_{5}$GMR$_{7}$, which does not associate with chromosomes, did not (Fig. 2F). Further, GST LANA 1-23 $_{17}$PLT$_{19}$ and GST LANA 1-23 $_{20}$RGS$_{22}$ bound purified nucleosomes and nucleosomes from BJAB cell extracts (Fig. 2G and fig. S2). In contrast, GST LANA 1-23 $_{5}$GMR$_{7}$, GST LANA 1-23 $_{8}$LRS$_{10}$, and GST LANA 1-23 $_{11}$GRS$_{13}$, substituted at residues essential for chromosome binding, did not bind histones (Fig. 2, E and G, and fig. S2). Full-length LANA substituted at residues essential for chromosome binding also did not bind core histones (fig. S3). GST LANA 1-23 $_{14}$TG$_{15}$ bound nucleosomes at a reduced amount (Fig. 2G and fig. S2), similar to the reduced chromosome binding with this mutation (Fig. 1B). Thus, the same LANA residues are critical for histone and chromosome binding, providing strong evidence that core histones mediate LANA chromosome attachment.

We next investigated through which histones the LANA N-terminal region binds nucleosomes. GST LANA 1-23 and GST were incubated with acid-extracted histones, which contain core histone H2A-H2B dimers and H3-H4 tetramers. GST LANA 1-23 precipitated histones H2A and H2B, but not H3 and H4 (Fig. 3A). GST did not bind histones. Antibody that detects both histones H1 and H2B confirmed the H2B binding and demonstrated that GST LANA 1-23 does not bind linker histone H1 (Fig. 3B). We also investigated whether LANA bound the tails or folded domain of H2A-H2B. GST LANA 1-23 precipitated both recombinant full-length H2A-H2B and tailless H2A-H2B (Fig. 3C). These results indicate that the LANA N terminus specifically binds nucleosomes through the folded domain of H2A-H2B.

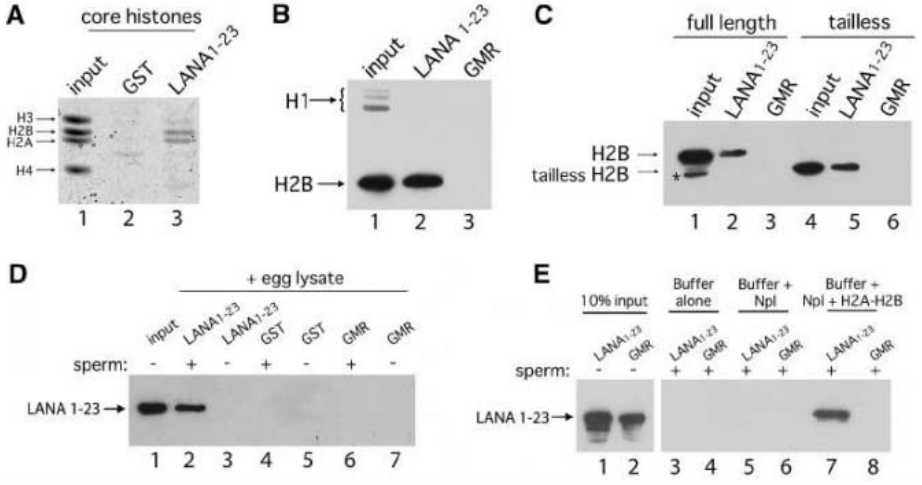

Fig. 3. Histones H2A-H2B are essential for LANA N-terminal chromosome binding. (**A**) GST or GST LANA 1-23 was incubated with purified histones, and bound histones were detected by Coomassie. Lane 1, 30% input. (**B**) GST LANA 1-23 or GST LANA 1-23 $_{5}$GMR$_{7}$ was incubated with purified histones, and precipitated H1 and H2B were detected by immunoblot. Lane 1, 30% input. (**C**) GST LANA 1-23 or GST LANA 1-23 $_{5}$GMR$_{7}$ was incubated with full-length or tailless H2A-H2B dimers, and precipitated H2B was detected by immunoblot. Input, 30%. Asterisk, degradation product. (**D**) GST, GST LANA 1-23, or GST LANA 1-23 $_{5}$GMR$_{7}$ was incubated in egg lysate HSS with or without *Xenopus* sperm chromatin, and chromatin-bound GST proteins were detected by immunoblot. Input, 10%. (**E**) GST LANA 1-23 or GST LANA 1-23 $_{5}$GMR$_{7}$ was incubated with *Xenopus* sperm chromatin in buffer alone, with purified nucleoplasmin (Npl), or with nucleoplasmin plus H2A-H2B dimers. Chromatin-bound GST proteins were detected.

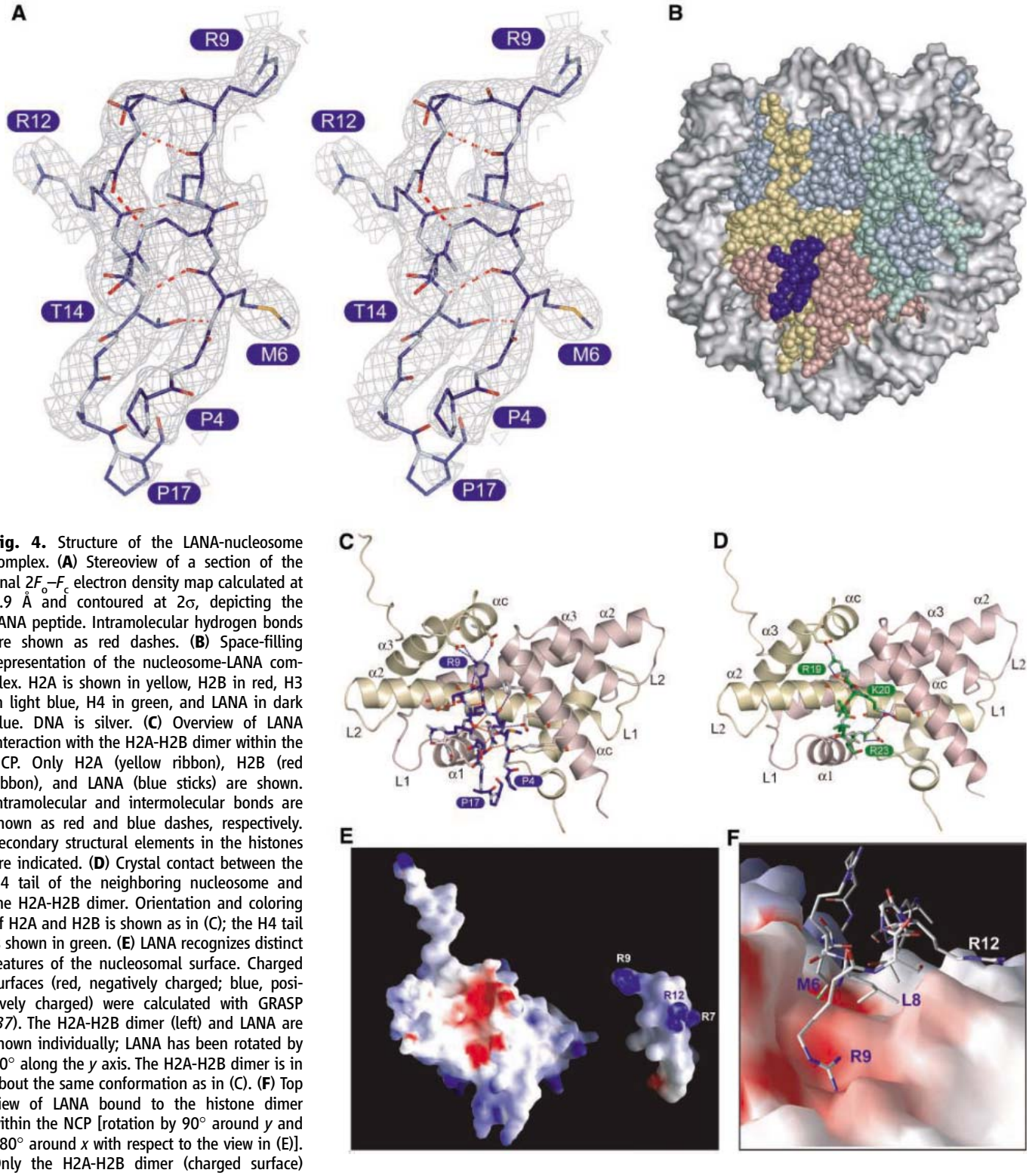

Fig. 4. Structure of the LANA-nucleosome complex. (**A**) Stereoview of a section of the final $2F_o–F_c$ electron density map calculated at 2.9 Å and contoured at 2σ, depicting the LANA peptide. Intramolecular hydrogen bonds are shown as red dashes. (**B**) Space-filling representation of the nucleosome-LANA complex. H2A is shown in yellow, H2B in red, H3 in light blue, H4 in green, and LANA in dark blue. DNA is silver. (**C**) Overview of LANA interaction with the H2A-H2B dimer within the NCP. Only H2A (yellow ribbon), H2B (red ribbon), and LANA (blue sticks) are shown. Intramolecular and intermolecular bonds are shown as red and blue dashes, respectively. Secondary structural elements in the histones are indicated. (**D**) Crystal contact between the H4 tail of the neighboring nucleosome and the H2A-H2B dimer. Orientation and coloring of H2A and H2B is shown as in (C); the H4 tail is shown in green. (**E**) LANA recognizes distinct features of the nucleosomal surface. Charged surfaces (red, negatively charged; blue, positively charged) were calculated with GRASP (*37*). The H2A-H2B dimer (left) and LANA are shown individually; LANA has been rotated by 90° along the *y* axis. The H2A-H2B dimer is in about the same conformation as in (C). (**F**) Top view of LANA bound to the histone dimer within the NCP [rotation by 90° around *y* and 180° around *x* with respect to the view in (E)]. Only the H2A-H2B dimer (charged surface) and LANA (stick model) are shown.

We wished to demonstrate directly that the LANA N terminus uses H2A-H2B to bind chromosomes. We used *Xenopus laevis* sperm chromatin, which is naturally deficient in H2A-H2B and instead contains sperm-specific basic proteins X and Y. In addition, *Xenopus* sperm lack H1 (*16*–*18*). Upon incubation with high-speed supernatant (HSS) from *Xenopus* egg lysate, egg cell–derived nucleoplasmin protein mediates sperm chromatin decondensation and replacement of X and Y with egg H2A-H2B dimers. To verify LANA chromosome binding in this system, we incubated HSS-treated chromatin, which contains wild-type H2A-H2B dimers, with GST fusions. GST LANA 1-23 bound sperm chromatin that had undergone H2A-H2B deposition through HSS treatment, but GST LANA 1-23 $_5$GMR$_7$ and GST did not (Fig. 3D). No LANA protein precipitated in the absence of chromatin. Therefore, N-terminal LANA binds *Xenopus* chromosomes after H2A-H2B deposition.

We stringently assayed whether H2A-H2B were required for LANA chromosome binding. HSS contains other factors in addition to H2A-H2B and nucleoplasmin. We therefore used a purified system with nucleoplasmin and recombinant H2A-H2B dimers in place of HSS. GST LANA 1-23 did not bind H2A-H2B-deficient sperm chromatin that had been treated with buffer or with purified nucleoplasmin alone. However, after incubation with nucleoplasmin and recombinant histone H2A-H2B dimers, which allows for deposition of histones H2A-H2B into sperm chromatin, GST LANA 1-23 specifically bound sperm chromatin (Fig. 3E). Thus, H2A-H2B is essential for LANA chromosome binding.

We solved the x-ray crystal structure of LANA residues 1 to 23 complexed with the NCP. Data collection and refinement statistics are summarized in table S1. Figure 4A shows a $2F_o–F_c$ map of the final model of the LANA peptide, contoured at 2σ. LANA forms a tight hairpin that is stabilized by five intramolecular hydrogen bonds (three β-type interactions and two side-chain or main-chain interactions) (Fig. 4, A and C) and by numerous hydrogen bonds and Van der Waals contacts with the nucleosomal surface.

Consistent with the biochemical experiments (Fig. 3, A to C), the LANA peptide interacts exclusively with the H2A-H2B dimer within the nucleosome (Fig. 4B). Histone fold regions and extensions of H2A and H2B are implicated in the interaction, but not the flexible histone tails. The hairpin is wedged between the αC and α1 helix of H2B (Fig. 4C); the turn of the hairpin abuts the H2A docking domain that forms a major interaction interface between the H2A-H2B dimer and the $(H3\text{-}H4)_2$ tetramer (*19*). The L1 loop of H2B as well as the α2 and α3 helices of H2A are also involved in LANA binding, consistent with the requirement for a folded H2A-H2B dimer for LANA binding. Molecular details of the interactions between LANA and the nucleosome are shown in fig. S4 (SOM Text). Substitution of individual LANA amino acids 5 to 16 demonstrated that residues important for chromosome association (fig. S5 and SOM Text) have critical roles in the interaction between LANA and the NCP. Of note, the overall structure of the nucleosome is maintained upon LANA binding (Fig. 4B).

Interactions of LANA with the NCP resemble those between the NCP and the H4 N-terminal tail from a neighboring nucleosome within the crystal lattice (Fig. 4D) (*20*). Both peptides interact with the same conserved acidic patch composed of several residues from H2A and H2B on the highly contoured nucleosomal surface (*21*). Despite a lack of sequence homology between the LANA peptide and the N-terminal tail, many of the targeted residues in H2A and H2B are the same (see, for example, LANA R_9 and H4 R_{19} in Fig. 4, C and D, respectively). The interaction shown in Fig. 4D is essential for nucleosome crystallization (*14*), and biophysical experiments have indicated a unique role for the H4 tail and acidic patch interaction in the formation of chromatin higher order structure (*22*, *23*).

Analysis of the molecular surfaces of both the LANA peptide and the H2A-H2B dimer demonstrates excellent shape and charge complementarity (Fig. 4E), indicating that the LANA N-terminal region has evolved to recognize this region within the NCP with high specificity. LANA R_9 and Ser_{10} point into the acidic pocket formed by H2A and H2B, and hydrophobic LANA residues are inserted deep into a cleft delineated by the αC helix of H2B (Fig. 4F). The LANA peptide interaction buries 1340 Å^2, well within the range that is considered to be a stable interaction (*24*), which is notable considering that only 14 residues of LANA contribute to the interaction. For comparison, the molecular surface buried by the H4 tail–NCP interaction (Fig. 4D) is only 680 Å^2 and contains larger cavities.

This work demonstrates that LANA's N-terminal chromosome association is mediated by H2A-H2B and not by the earlier proposed candidates methyl-CpG binding protein 2 (MeCP2) or H1 (*8*, *12*, *25*). It was previously reported that LANA did not associate with murine chromosomes unless human MeCP2 was co-expressed (*8*). In contrast, we found that LANA bound murine chromosomes (fig. S1); further, MeCP2 was not identified from our affinity purification. Histone H1 did not bind the LANA N terminus and was not required for LANA to bind *Xenopus* chromatin (Fig. 3, B and E). These results also differ from proposed chromosome binding mechanisms for other episome maintenance proteins: Epstein-Barr virus EBNA1 binds chromosomes through the nucleolar EBP2 protein or AT hooks, and bovine papillomavirus E2 binds through the bromodomain protein Brd4 (*26*–*28*).

This work may also link H2A-H2B binding to LANA's transcriptional regulatory effects (*29*, *30*). In fact, LANA transcriptional activity can be dependent on N-terminal chromosome association (*31*). An intriguing possibility is that LANA may affect transcription by regulating transient H2A-H2B removal from nucleosomes through complexes such as FACT or nucleosome assembly protein 1 (*32*, *33*). Histone modifications regulate transcription and may also affect LANA's affinity for nucleosomes and effects on chromatin, although experiments with bacterially expressed protein (Figs. 2 to 4) indicate that histone modifications are not required for binding.

This work indicates a role for H2A-H2B in LANA-mediated DNA replication and episome persistence, because these functions are dependent on N-terminal LANA chromosome binding (*7*). Interestingly, histone fusions have been used as an alternative method of targeting LANA and EBNA1 to chromosomes (*10*, *27*, *34*, *35*). Linker histone H1 in place of the LANA or EBNA1 chromosome association region permits episome persistence, whereas core histones (H2B and H3, respectively) do not, perhaps because of positional restrictions related to the covalent linkages. Of note, LANA has a C-terminal chromosome association domain, but it cannot rescue chromosome binding of N-terminal mutated LANA (Fig. 1B) (*7*–*10*); its role in episome persistence is currently under investigation. The distribution of H2A-H2B throughout chromosomes provides a platform through which LANA tethered episomes can efficiently segregate to progeny nuclei. Strategies that interrupt the interaction between LANA and H2A-H2B may provide effective treatment and prevention of KSHV-associated diseases.

The x-ray crystal structure shows that a hairpin formed by KSHV LANA residues 5 to 13 interacts with eukaryotic chromatin by binding to an acidic patch formed by H2A-H2B within the nucleosome. Thus, LANA has evolved to use the differentially charged and contoured surface of the nucleosome as a "docking station" for episome attachment. The concept of the nucleosomal surface (as opposed to the flexible histone tails) as an interaction platform has been proposed earlier (*14*, *22*, *36*–*38*); we now report the structure of a protein complexed with the nucleosome core. It appears that an important function of histones, in addition to maintaining interaction with other histones to form the octamer and compacting genomic DNA, is to maintain a distinct surface landscape that is used as a docking platform by cellular and viral factors. Such interactions may locally affect nucleosome dynamics and/or alter chromatin higher order structure, with profound implications for transcription of underlying DNA regions.

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Materials and Methods
SOM Text
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Neurochemical Modulation of Response Inhibition and Probabilistic Learning in Humans

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Cognitive functions dependent on the prefrontal cortex, such as the ability to suppress behavior (response inhibition) and to learn from complex feedback (probabilistic learning), play critical roles in activities of daily life. To what extent do different neurochemical systems modulate these two cognitive functions? Here, using stop-signal and probabilistic learning tasks, we show a double dissociation for the involvement of noradrenaline and serotonin in human cognition. In healthy volunteers, inhibition of central noradrenaline reuptake improved response inhibition but had no effect on probabilistic learning, whereas inhibition of central serotonin reuptake impaired probabilistic learning with no effect on response inhibition.

Ascending monoamine projections play important neuromodulatory roles in high-level cognition through actions upon the prefrontal cortex (PFC), a major brain structure with considerable functional heterogeneity in humans (*1*). Dysfunction in these neurochemical systems is implicated in the etiology and psychopathology of psychiatric illnesses associated with cognitive deficits and PFC abnormalities, including depression, attention deficit–hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), and drug addiction (*2*–*7*). Dopamine regulates executive functions dependent on the dorsolateral PFC, including working memory and attentional set-shifting, but the role of noradrenaline (NA) and serotonin [5-hydroxytryptamine (5-HT)] in cognition is less well characterized (*8*). The orbitofrontal cortex (OFC) is involved in emotion-cognition interactions, and 5-HT drugs modulate response to feedback and decision-making within this region (*9*–*15*). 5-HT and NA have both been implicated in response inhibition (*16*, *17*), a function that has been linked to the right inferior frontal gyrus (RIFG) (*18*).

We investigated the differential involvement of NA and 5-HT transmitter systems in these processes in humans, using the selective NA reuptake inhibitor (SNRI) atomoxetine and the selective 5-HT reuptake inhibitor (SSRI) citalopram. These agents are among the most selective inhibitors for brain NA and 5-HT reuptake transporters available for human use, according to in vitro and in vivo findings (*19*–*21*). Microdialysis studies in experimental animals have shown that acute systemic administration of atomoxetine rapidly increases PFC NA but not 5-HT and that the administration of citalopram rapidly increases PFC 5-HT but not NA (*19*, *22*). As such, these agents represent useful neurochemical tools for investigating the differential involvement of NA and 5-HT in human cognition.

Response inhibition, the ability to exert high-level inhibitory control over motor responses so as to suppress unwanted actions, can be assessed with the stop-signal procedure (*6*, *23*). In this procedure, volunteers are required to make rapid motor responses on Go trials but to inhibit responses if an auditory stop-signal occurs. By the infrequent nature of Stop trials, motor responses are made "prepotent." Response inhibition can be quantified by the stop-signal reaction time (SSRT), an estimate of the time taken to inhibit the prepotent motor response (*18*, *23*). Probabilistic learning refers to the ability to develop cognitive associations between stimuli and outcomes on the basis of punishing and rewarding feedback, and to modify these associations as appropriate (*12*). On probabilistic learning tasks, volunteers are required to select which of two stimuli they believe to be correct over a series of trials. After each choice, the computer provides punishing or rewarding feedback that is "degraded" (i.e., misleading on a subset of trials) (*12*).

The aim of the present study was to delineate the precise differential contribution of NA and 5-HT neurochemical systems to response inhibition and probabilistic learning. Sixty healthy male participants were recruited from the local community on the basis of being free from medical or psychiatric disorders according to assessment by a psychiatrist (mean age 25.7 ± SD 4.7 years, range 20 to 35) (*24*). Participants received single clinically relevant oral doses of atomoxetine (60 mg), citalopram (30 mg), or placebo in a double-blind parallel-groups design (*24*). Groups were matched for demographic characteristics (table S1). After spending 1.5 hours in a quiet waiting area to ensure drug

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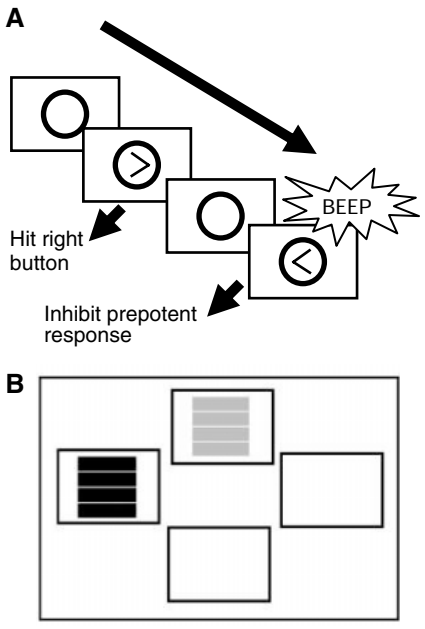

Fig. 1. (**A**) On the computerized stop-signal task, subjects respond rapidly to left- or right-facing arrows on screen with corresponding motor responses, and they attempt to inhibit responses when an auditory stop-signal sounds. Over the course of the task, the time between stimulus onset and occurrence of the stop-signal is varied by means of a tracking algorithm. This permits calculation of the SSRT, which reflects an estimate of the time taken to internally suppress prepotent motor responses [for further details of calculation, see (*18*, *23*)]. The average response time for Go trials is also recorded. (**B**) On the probabilistic learning task, volunteers make a two-alternative forced choice between two stimuli (one red, one green) on each trial. The "correct" stimulus (always the first stimulus touched) receives an 8:2 ratio of positive:negative feedback, and the opposite ratio is given for the "incorrect" stimulus. Feedback is provided in the form of "CORRECT" or "INCORRECT" appearing on screen after each choice. Ability to acquire the stimulus-reward association on the basis of this degraded feedback is assessed by the number of errors made before reaching criterion, defined as eight consecutive correct responses to the maximally rewarded stimulus. After 40 trials (stage 1), the contingencies reverse for the subsequent 40 trials (stage 2) (i.e., if "red" was previously correct, then "green" becomes correct). Ability to reverse the previously acquired stimulus-reward association is assessed by the number of perseverative errors to the previously maximally rewarded stimulus. Ability to acquire the new stimulus-reward association is again assessed by the number of errors made before reaching criterion. The detrimental effect of misleading negative feedback on learning is assessed by means of an overall "feedback sensitivity" score. This is defined as the overall likelihood that the volunteer inappropriately switched to choose the incorrect stimulus after misleadingly being informed that his or her correct response on the previous trial was not correct.

absorption, volunteers completed the stop-signal and probabilistic learning tasks (Fig. 1).

The results from the two tasks are shown in Fig. 2. The citalopram-treated group did not differ from controls in terms of response inhibition, but the atomoxetine-treated group showed shorter SSRTs (i.e., superior response inhibition) relative to both of the other groups. On the probabilistic learning task, the performance of the citalopram-treated volunteers was impaired on several measures, whereas the performance of the atomoxetine-treated group did not differ from that of the placebo group. The citalopram-treated group made increased numbers of errors before achieving learning criterion, were slower to respond, and were more likely to shift responding away from the correct stimulus after receiving misleading feedback.

These findings show that response inhibition and probabilistic learning are separable cognitive functions that are differentially modulated by ascending monoamine systems. Response inhibition was enhanced by inhibition of central NA reuptake but was unaffected by inhibition of central 5-HT reuptake. Conversely, probabilistic learning was impaired by inhibition of central 5-HT reuptake but was unaffected by inhibition of central NA reuptake. We excluded a nonspecific influence of atomoxetine

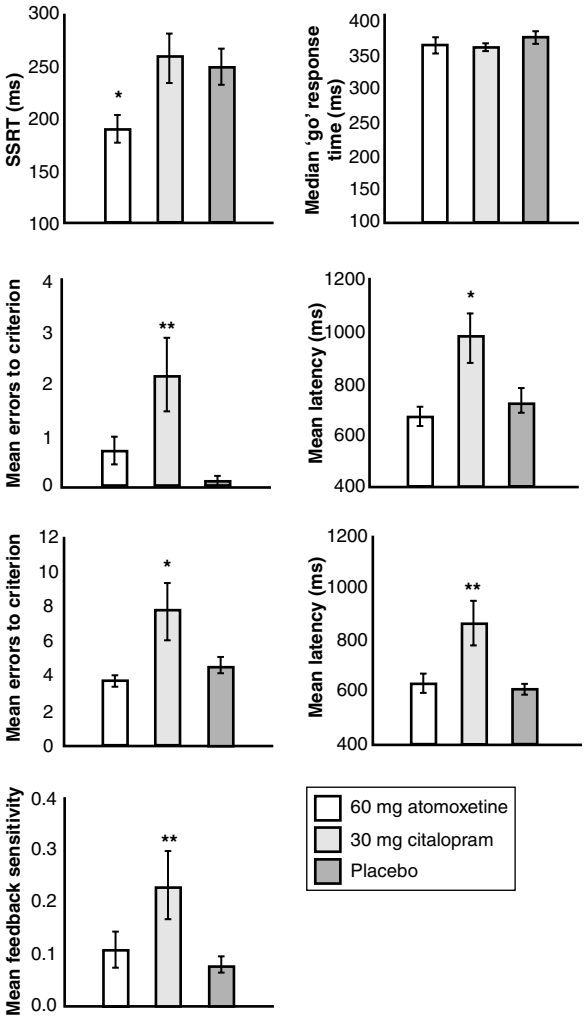

Fig. 2. Atomoxetine enhances response inhibition on the stop-signal task, whereas citalopram impairs performance on the probabilistic learning task. *$P < 0.05$ difference versus controls; **$P < 0.01$ difference versus controls. Error bars show SEM. In the stop-signal task, groups differed significantly on SSRTs ($F_{2,57} = 4.377$, $P = 0.017$) but not on median Go response times ($F_{2,57} = 0.780$, $P = 0.463$). The atomoxetine-treated group showed significantly shorter SSRTs relative to the citalopram-treated group ($P = 0.013$) and the placebo group ($P = 0.014$), whereas the citalopram-treated group did not differ from the placebo group ($P = 0.973$). In the probabilistic learning task, groups in stage 1 (graphs, second row) differed overall on number of errors made before attaining criterion ($F_{2,57} = 5.549$, $P = 0.006$) and on response latency ($F_{2,57} = 5.588$, $P = 0.006$). The citalopram-treated group made more errors before reaching criterion than did the atomoxetine-treated group ($P = 0.012$) and the placebo group ($P = 0.002$) and displayed longer mean response latencies than did the atomoxetine-treated group ($P = 0.003$) and the placebo group ($P = 0.012$). The atomoxetine-treated group did not differ from the placebo group on these measures ($P = 0.379$; $P = 0.616$). In stage 2, groups did not differ significantly in terms of perseverative errors made to the previously maximally rewarded stimulus (mean errors ± SD: atomoxetine, 2.95 ± 1.67; citalopram, 4.00 ± 6.54; placebo, 3.15 ± 1.73; $F_{2,57} = 0.390$, $P = 0.679$). Groups differed overall (graphs, third row) on number of errors made before attaining criterion ($F_{2,57} = 5.019$, $P = 0.010$) and on response latency ($F_{2,57} = 7.981$, $P = 0.001$). The citalopram-treated group made more errors before reaching criterion than did the atomoxetine-treated group ($P = 0.004$) and the placebo group ($P = 0.020$) and displayed longer mean response latencies than did the atomoxetine-treated group ($P = 0.001$) and the placebo group ($P = 0.001$). The atomoxetine-treated group did not differ from the placebo group on these measures ($P = 0.557$; $P = 0.885$). Groups differed on feedback sensitivity scores ($F_{2,57} = 4.109$, $P = 0.022$). The citalopram-treated group showed greater feedback sensitivity than did the atomoxetine-treated group ($P = 0.037$) and the placebo group ($P = 0.009$). The atomoxetine-treated group did not differ from the placebo group on this measure ($P = 0.554$).

and citalopram on attentional function or arousal. There were no significant effects of either drug on subjective rating scales for factors of alertness, contentedness, or calmness (*24*). There were also no effects of drug on a sensitive background test of sustained attention (table S2) or on the median Go reaction time on the stop-signal procedure. As a double dissociation was observed, these data strongly support the proposal that NA and 5-HT play distinct roles in the control of response inhibition and probabilistic learning. These results have important implications for our understanding of coupling between neurochemical systems and PFC processing.

Response inhibition is critically dependent on the PFC. ADHD patients show impaired response inhibition alongside abnormalities in the RIFG, according to structural and functional neuroimaging investigations (*6*, *25*). Further, patients with lesions of the right PFC show impaired response inhibition that correlates with the degree of volume loss (*18*). The finding that atomoxetine improved response inhibition in these healthy volunteers implicates ascending NA systems in its control. Inhibition of 5-HT reuptake had no effect on response inhibition, consistent with previous work showing that depletion of central 5-HT likewise had no effect on response inhibition in healthy volunteers (*26*) and contradicting the simple hypothesis of 5-HT involvement in behavioral inhibition (*16*).

Our findings are important in relation to current treatment algorithms for ADHD, in which problems with response inhibition have been argued to represent a core cognitive deficit (*6*). Although the evidence to date does not support the utility of 5-HT drugs in mitigating core cognitive symptoms of this disorder, atomoxetine and psychostimulant medications such as methylphenidate are known to be effective and to act via mechanisms involving NA and/or dopamine (*27*, *28*). Atomoxetine augments PFC NA and may also alter PFC dopamine levels via actions on NA reuptake transporters (*19*, *29*). However, bilateral infusion of the α2-adrenoceptor blocker yohimbine into monkey PFC has been shown to impair response inhibition (*30*), whereas in humans, yohimbine and desipramine (a nonselective NA reuptake inhibitor) impair and improve response inhibition, respectively (*17*, *31*). Administration of L-dioxyphenylalanine (L-DOPA), acting predominantly on dopaminergic mechanisms, has no effect on response inhibition in children with ADHD (*17*) and has limited efficacy in treating clinical symptoms (*17*, *27*). Therefore, the most parsimonious explanation of the beneficial effects of atomoxetine is that it enhances stopping selectively via actions on NA uptake. Despite the traditional association between impulse control disorders and abnormal 5-HT transmission (*16*, *32*), NA drugs may be more suited to ameliorating impaired response inhibition as a therapeutic target (*28*).

Multiple tiers of evidence implicate the PFC (particularly the OFC) in affective processing, in the establishment of stimulus-outcome contingencies, and in the deployment of this information to guide behavior (*12*, *33*–*36*). Consistent with a neuromodulatory role for 5-HT in probabilistic learning, acute administration of citalopram exerted deleterious effects on probabilistic learning in healthy volunteers. Similar impairments in feedback learning have been demonstrated in depression and in studies of 5-HT depletion in healthy volunteers (*10*, *12*, *15*, *37*, *38*). One possible explanation for the deleterious effects of citalopram reported here is that presynaptic autoreceptor feedback effects may have induced a temporary reduction in 5-HT function after acute citalopram dosing (*39*, *40*). An alternative account is that the relationship between 5-HT neurotransmission and probabilistic learning operates according to an "inverted-U" function, whereby either underactivity or overactivity of a neurotransmitter system can impair cognition (*8*, *41*). As such, citalopram may have caused supraoptimal PFC 5-HT availability. The effects of citalopram on probabilistic learning may differ in psychiatric disorders associated with functionally abnormal 5-HT systems (such as OCD and depression), and contrasting the effects of acute and chronic SSRI treatment would help to further elucidate the neuropsychological mechanisms by which SSRIs exert their beneficial treatment effects.

This study has provided theoretically important evidence, in normal subjects, for the modulation of distinct cognitive functions after acute selective NA and 5-HT reuptake blockade. The findings also have clinical implications in the context of the treatment by atomoxetine of response inhibition deficits manifested in ADHD, and in understanding the effects of SSRI treatment on the cognitive sequelae of OCD and depression.

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Supporting Online Material

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Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress

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Mice experiencing repeated aggression develop a long-lasting aversion to social contact, which can be normalized by chronic, but not acute, administration of antidepressant. Using viral-mediated, mesolimbic dopamine pathway–specific knockdown of brain-derived neurotrophic factor (BDNF), we showed that BDNF is required for the development of this experience-dependent social aversion. Gene profiling in the nucleus accumbens indicates that local knockdown of BDNF obliterates most of the effects of repeated aggression on gene expression within this circuit, with similar effects being produced by chronic treatment with antidepressant. These results establish an essential role for BDNF in mediating long-term neural and behavioral plasticity in response to aversive social experiences.

The mesolimbic dopamine pathway, composed of dopaminergic neurons in the midbrain ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc), allows an organism to identify emotionally salient stimuli in the environment, to learn about outcomes associated with those stimuli, and to express appropriate approach or avoidance responses (*1*, *2*). Activation of this neural circuit has been characterized extensively in relation to drugs of abuse but has been less characterized in ethologically relevant contexts (*3*–*5*). The circuit is stimulated in humans and animals by psychosocial experiences such as affiliation and cooperation (*6*, *7*), and it drives associative learning processes such as imprinting, pair bonding, and maternal attachment (*8*, *9*). Aversive stimuli such as aggression and social subordination (*10*) also acutely activate the mesolimbic dopamine pathway (*11*–*13*) and have been linked to chronic alterations in dopaminergic function (*14*). These observations have led to the hypothesis that dopaminergic signaling to the NAc may be involved in the perception of social status and the appraisal of threats from the social environment (*8*). Imaging studies have linked the NAc to cognitive processes that lead to the attribution of salience to social stimuli (*15*). Alteration of this cognitive function could contribute to a social withdrawal trait that is common to several human affective disorders, including depression, social phobia, and post–traumatic stress disorder (PTSD), in which dopaminergic abnormalities have been described (*16*–*19*). However, very little is known about the mechanisms through which the motivational value of socially relevant stimuli might be encoded by this pathway.

To characterize the neurobiological mechanisms through which psychosocial experience alters the activity of the mesolimbic dopamine pathway, we adopted a social defeat paradigm that profoundly alters the motivation for social interactions in rodents (*20*–*22*). Mice were subjected to daily bouts of social defeat, followed by continuous protected sensory contact with their aggressor [Fig. 1A; see fig. S1 in the supporting online material (SOM) for experimental details]. Mice were exposed to a different aggressor each day for 10 days and were then screened for social behavior. We measured social approach toward an unfamiliar mouse enclosed in a wire mesh cage by use of a video-tracking system (Fig. 1B). Undefeated control mice spent most of their time interacting socially when presented with an unfamiliar target mouse. Defeated mice displayed intense aversive responses and spent less time in close proximity to the target mouse (Fig. 1, B and C). This difference was observed exclusively in the presence of a social target and was not apparent in response to an inanimate novel object [the empty wire cage (Fig. 1B)]. No difference in total movement in the arena was observed (Fig. 1C). When tested again 4 weeks after the 10 days of repeated psychosocial stress, mice with a history of social defeat still displayed dramatic social avoidance (Fig. 1D). This aversive response was more robust when a former aggressor was used as a social target in the wire cage, but it also generalized to unfamiliar mice that were physically distinct from the aggressors (fig. S2). To test whether this long-lasting change in social behavior is relevant to stress-linked human conditions characterized by social withdrawal, we studied the effect of antidepressants in our model. Chronic, but not acute, administration of fluoxetine or imipramine, two chemically distinct antidepressants used widely in humans, improved social interaction in defeated animals (Fig. 2A), an effect that could not be explained by changes in general locomotion (fig. S3). This effect was not produced by acute or chronic (fig. S4) treatment with chlordiazepoxide, a benzodiazepine used to treat anxiety but not depression in humans.

We next measured c-Fos expression in the VTA and NAc as a marker of neuronal activation after sensory exposure to social cues. We found robust c-Fos induction in VTA dopamine neurons and in their target neurons in the NAc when mice were exposed to a social partner through a perforated Plexiglas partition (fig. S5). As compared to naïve mice, defeated mice exhibited sensitized c-Fos responses when exposed to a social target 4 weeks after defeat (Fig. 1E).

The neurotrophic factor BDNF (brain-derived neurotrophic factor) is a key regulator of the mesolimbic dopamine pathway. BDNF potentiates dopamine release in the NAc through activation of TrkB receptors on dopaminergic nerve terminals (*23*), and it potently regulates NAc function directly via activation of TrkB receptors on NAc neurons (*24*–*26*). We hypothesized that BDNF function within the mesolimbic dopamine pathway may be a critical mediator of changes in social motivation. We found that 10 days of social defeat increased BDNF protein levels in the NAc, an effect that was apparent both 24 hours and 4 weeks after the stress (Fig. 1F).

A major source of BDNF protein in the NAc is thought to be the VTA (*26*), because BDNF mRNA is expressed at high levels by dopaminergic neurons (*27*, *28*) but is barely detectable in NAc neurons (*29*). We thus induced a local deletion of the gene encoding BDNF that was restricted to VTA neurons in adult mice. We used a line of mice in which 1 kb of the single coding exon of the BDNF gene is flanked by loxP sites (floxed BDNF mice) (Fig. 3A). We injected adult mice with an adenoassociated virus (AAV) vector expressing green fluorescent protein (GFP)–tagged Cre recombinase (CreGFP) or with GFP as a control directly into the VTA (*30*). Animals were subjected to the social defeat paradigm 20 days after the infusion, when AAV-CreGFP–induced recombination is maximal (see methods in the SOM). Local deletion of the BDNF gene exerted an antidepressant-like effect by opposing the development of social avoidance behavior in defeated mice (Fig. 2B). Defeated mice injected with AAV-GFP showed the expected social avoidance behavior. Control mice injected with AAV-CreGFP were indistinguishable from AAV-

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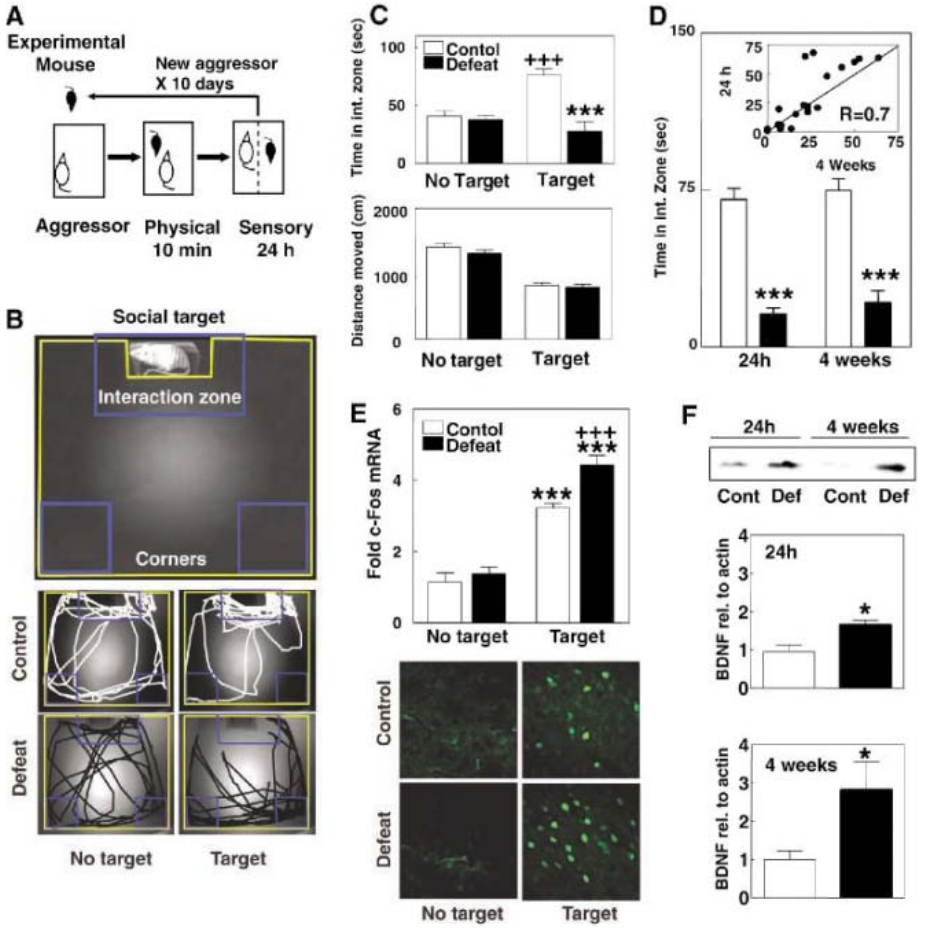


Fig. 1. Persistent social aversion after repeated aggression in mice. (**A**) Social defeat paradigm. (**B**) Videotracking data from control and defeated mice in the absence and presence of a social target. (**C**) (Top) A social target increased the time spent in the interaction zone by control mice (n = 18 mice), an effect suppressed after social defeat (n = 36 mice), which had no effect on total locomotion (bottom). [Analysis of variance (ANOVA): Target × defeat interaction F (2,150) = 7.26, $P < 0.001$. Least-square difference (LSD) post-hoc test: +++$P < 0.001$ versus control "no target," ***$P < 0.001$ versus "target."] (**D**) Stable social avoidance in defeated mice tested 24 hours and 4 weeks after 10 days of defeat stress (n = 13 or 14 mice). [Effect of social defeat F (1,60) = 115.5, $P < 0.0001$; no significant effect of test days, F (1,60) = 0.94, P = 0.33; significant correlation at 24 hours and 4 weeks, Pearson R = 0.696, $P < 0.001$]. (**E**) Sensitized c-Fos induction in the NAc of mice 4 weeks after repeated social defeat upon exposure to a social target. [ANOVA: Target × defeat interaction F (1,21) = 4.62, $P < 0.05$. LSD post-hoc test: +++$P < 0.001$ versus control "target" ***P <0.001 versus "no target," n = 6 or 7 mice.] (**F**) Increased BDNF protein levels in the NAc 24 hours and 4 weeks after 10 days of defeat stress [t(8) = –3.22, *$P < 0.05$, n = 5 mice]. Cont., control; def., defeat.

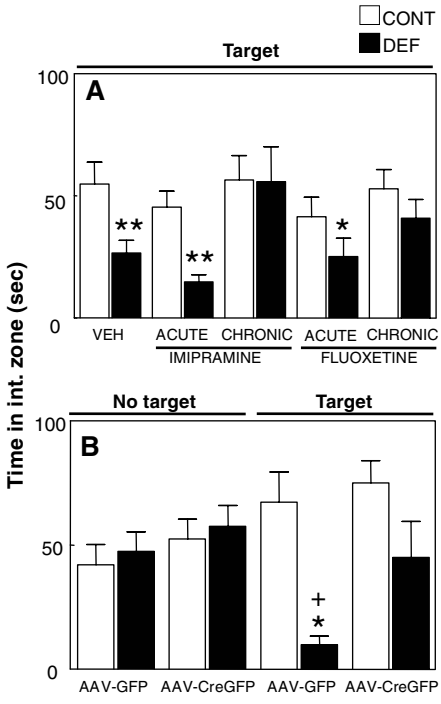


Fig. 2. Chronic treatments with antidepressant or VTA-specific deletion of BDNF oppose the development of social aversion after defeat stress. (Int. zone, interaction zone; veh., vehicle.) (**A**) Administration of imipramine [20 mg per kg of body weight (mg/kg)] or fluoxetine (20 mg/kg) daily for 28 days (chronic), but not a single injection (acute), reduced social avoidance caused by defeat stress. [ANOVA, significant effect of social defeat F (1,196) = 7.51, $P < 0.01$. Significant effect of antidepressants F (4,196) = 5.01, $P < 0.001$. LSD post-hoc test: *$P < 0.05$ and **$P < 0.01$ versus control, n = 9 to 18 mice.] (**B**) Social avoidance caused by defeat stress in floxed-BDNF mice injected with AAV-GFP in the VTA was reduced in mice injected with AAV-CreGFP [ANOVA: significant effect of virus, F (1,86) = 5.17, $P < 0.05$; significant target × defeat interaction, F (1,86) = 12.27, $P < 0.001$. LSD post-hoc test: +$P < 0.05$ versus AAV-GFP "no target," *$P < 0.05$ versus AAV-Cre "target," n = 10 to 13 mice.]

GFP–injected or uninjected controls (Fig. 2B). This indicates that the effect of BDNF deletion on social behavior is experience-dependent and requires repeated exposure to an aggressor. Therefore, in defeated mice, BDNF from VTA neurons is required for a social target to progressively acquire salience as a threatening stimulus. This associative process implicates a form of activity-dependent neuronal plasticity in the VTA-NAc pathway, which is mediated by BDNF. Indeed, c-Fos co-immunolabeling with tyrosine hydroxylase and GFP indicated that a significant proportion of the neurons infected by the AAV-CreGFP vector in the VTA were activated as a consequence of exposure to social cues (Fig. 3E).

Several lines of evidence confirm the efficiency of Cre-induced recombination in VTA dopaminergic neurons in our assay. Adult Rosa26 mice, in which recombination induces the *lacZ* gene, were injected with AAV-CreGFP or AAV-GFP in the VTA. Both vectors induced robust GFP expression in the VTA, but β-galactosidase (β-Gal) expression (evidence of recombination) was seen only in the AAV-CreGFP–injected mice (fig. S6). This β-Gal expression was seen solely within Cre-expressing cells, which were largely dopaminergic (a conclusion based on colabeling with tyrosine hydroxylase). Roughly 75% of all dopaminergic neurons were infected under our conditions (Fig. 3D). Floxed-BDNF mice injected with AAV-CreGFP showed a selective loss of BDNF expression within the VTA as determined by double-labeling in situ hybridization (Fig. 3B). Analysis of VTA tissue by real-time polymerase chain reaction (PCR) showed that AAV-CreGFP induces recombination of the BDNF gene (Fig. 3A) and that this causes an almost complete loss of BDNF mRNA expression (Fig. 3C). In contrast, the loss of BDNF had no effect on the expression of tyrosine hydroxylase. This finding, together with the observation that CreGFP had no effect on the total number of dopaminergic neurons (Fig. 3D), indicates no loss of these neurons in the VTA after BDNF suppression. This is in contrast to previous reports, which found loss of substantia nigra dopamine neurons upon

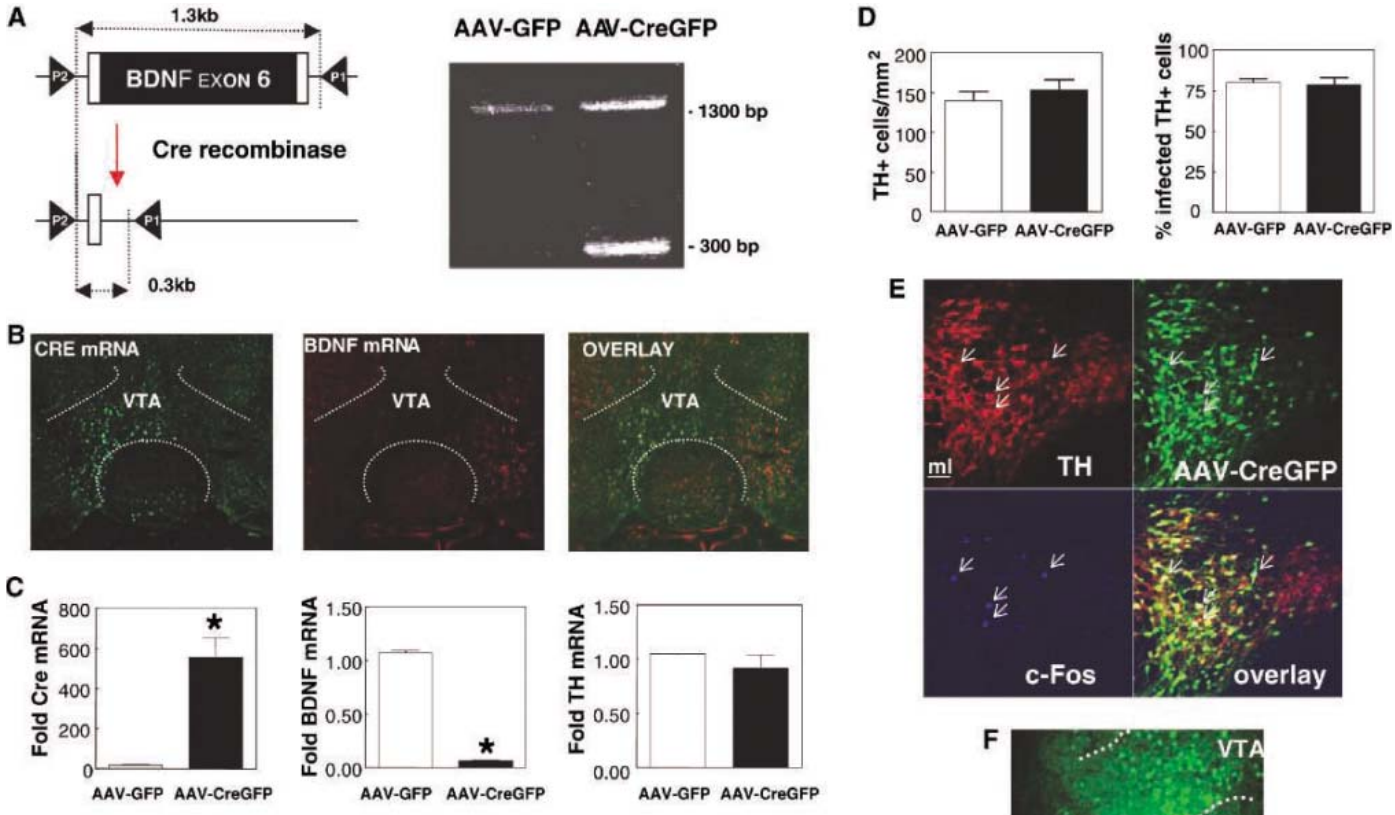


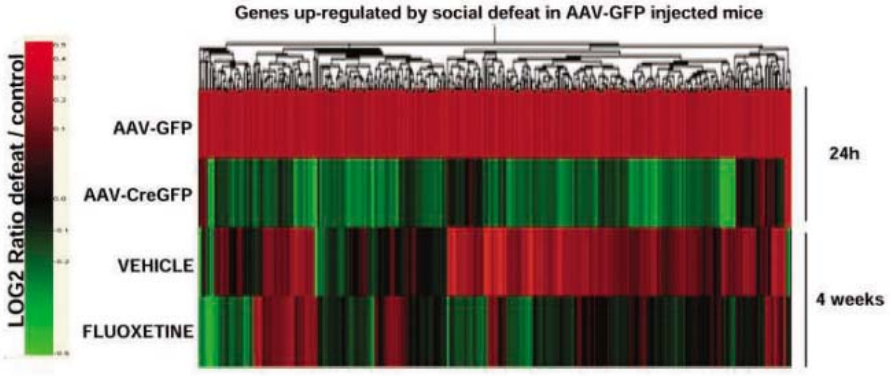
Fig. 3. AAV-CreGFP–mediated deletion of BDNF from VTA dopaminergic neurons. (**A**) (Left) Floxed-BDNF construct. (Right) PCR amplification of genomic DNA using primers encompassing the floxed exon (black triangles) demonstrated AAV-CreGFP–induced recombination in the VTA: A new 300–base pair (bp) fragment, the identity of which was confirmed by sequencing. (**B**) Double-labeling in situ hybridization of Cre recombinase and BDNF mRNAs in the VTA. Twenty days after unilateral infusion of AAV-CreGFP, BDNF mRNA was absent from the infected area where Cre expression was detected but was still present in the contralateral side. (**C**) Real-time PCR quantification of Cre, BDNF, and tyrosine hydroxylase (TH) mRNAs from laser-microdissected samples (F). AAV-CreGFP in the VTA caused an almost complete suppression of BDNF expression [$t(3) = -3.94$, $P < 0.05$, $n = 2$ or 3 mice], with no change in TH [$t(3) = 0347$, NS]. (**D**) AAV-CreGFP infected ~75% of TH+ cells in the VTA (right) but did not alter their total number (left). (**E**) Induction of c-Fos in AAV-infected dopaminergic neurons demonstrated by triple immunolabeling for TH, GFP, and c-Fos in the VTA of mice exposed to a social cue. The viral infection is contained within the VTA, which is medial to the medial lemiscus (ml). (**F**) Example of brain section before (top) and after (bottom) laser microdissection of AAV-infected cells from the VTA.

suppression of BDNF using early postnatal Cre expression (*28*) or the infusion of antisense oligonucleotides (*31*). The lack of dopaminergic neuronal loss seen in our study could reflect the different methodologies used or the lower sensitivity of VTA dopamine neurons to neurotoxic insults (*32*).

To gain further insight into the molecular events underlying the similar regulation of social defeat by BDNF gene deletion and antidepressant treatment, we carried out DNA microarray studies of gene expression in the NAc. In one experiment, mice received intra-VTA injections of AAV-CreGFP or AAV-GFP; half were then subjected to 10 days of social defeat stress; and 24 hours later, all mice were analyzed for NAc gene expression. In a parallel experiment, control or defeated mice received fluoxetine or vehicle for 4 weeks after chronic defeat stress. In mice injected with AAV-GFP and analyzed 24 hours after the end of the stress procedure, social defeat up-regulated 309 genes in the NAc, as compared to nondefeated mice, whereas 17 genes were down-regulated (Fig. 4A and tables S1 and S2). A similar pattern of gene expression, with 127 genes up-regulated and 9 genes down-regulated, was still observed 4 weeks after the cessation of social defeat in mice receiving vehicle injections (Fig. 4A and tables S1 and S2). After a discrete period of psychosocial stress, NAc neurons thus showed an activated pattern of gene expression, which persisted, like the change in social behavior, for up to 4 weeks. This heightened transcriptional activity may participate in encoding the motivational changes induced by aggression.

After local deletion of BDNF in the VTA, the effect of social defeat on most of these genes in the NAc was lost or reversed (Fig. 4A and tables S1 and S2). Chronic fluoxetine treatment also reversed most of the gene expression changes that persisted in the NAc after 4 weeks (Fig. 4A). Figure S7 provides examples of genes showing this pattern of expression in the NAc. Analysis of the regulated genes revealed specific molecular pathways induced prominently by defeat stress and reversed by BDNF deletion or fluoxetine treatment (fig. S8 and table S3). The largest subset of these regulated genes function in BDNF signaling cascades [such as phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (table S3 and figs. S8 and S9)] (*33*). The identification of PI3K in the NAc as an interface between the effects of social defeat and fluoxetine is particularly interesting because of its reported influence on dopamine release (*23*) and motivational processes (*34*, *35*). These microarray data thereby suggest that chronic treatment with antidepressant restores social approach behaviors partly by interfering with the activity of neurotrophic cascades that mediate experience-induced neuroadaptations in the mesolimbic dopamine pathway. Al-

A



B

	BDNF KO	FLUOXETINE
DOPAMININERGIC SYSTEM		
Dopa decarboxylase	+	
Dopamine receptor 2	+	
Dopamine receptor D1A	+	
GROWTH FACTORS AND GROWTH FACTOR SIGNALING		
Fibroblast growth factor (acidic) intracellular binding protein	+	
Fibroblast growth factor receptor 1	+	
Fibroblast growth factor receptor 3		+
Glycogen synthase kinase 3 beta	+	
Insulin-like growth factor 2 receptor	+	
Insulin-like growth factor binding protein 1	+	
Thymoma viral proto-oncogene 1 (AKT1)		+
Thymoma viral proto-oncogene 2 (AKT2)		+
Thymoma viral proto-oncogene 3 (AKT3)	+	
MAD3 homolog (TGF signaling)	+	+
Nerve growth factor, beta	+	
Mitochondrial tumor suppressor 1	+	+
CELL ADHESION AND MOTILITY		
Integrin alpha 6	+	
Integrin beta 4	+	
Integrin beta1	+	+
Non-catalytic region of tyrosine kinase adaptor protein 2	+	+
Dedicator of cytokinesis 9	+	+
Chemokine (C-C motif) receptor 4	+	
ADAM10	+	
ADAM11		+
ADAMTS-like 3	+	
Kinesin family member 1A		+
Kinesin family member 1B	+	
AP2 associated kinase 1	+	+
Beta-2 microglobulin	+	+
Unc-5 homolog C (axon guidance)	+	+
CYTOSKELETON	+	
Microtubule-actin crosslinking factor 1	+	
Actin-related protein 3-beta	+	+
Procollagen-proline alpha 1 polypeptide	+	+

	BDNF KO	FLUOXETINE
SIGNAL TRANSDUCTION		
cAMP pathway		
cAMP responsive element binding protein 1 (CREB1)		+
cAMP responsive element modulator	+	
Cbp/p300-interacting transactivator	+	
Phosphodiesterase 4B	+	
Phosphodiesterase 8B	+	+
CREB binding protein	+	
Cyclic AMP-regulated phosphoprotein, 21	+	
Map Kinase pathway		
Mitogen-activated protein kinase kinase 5	+	
Mitogen-activated protein kinase kinase 7	+	
Mitogen-activated protein kinase kinase kinase 10	+	
Mitogen-activated protein kinase kinase kinase 3	+	
Mitogen-activated protein kinase kinase 1 interacting protein 1	+	
Mitogen-activated protein kinase kinase kinase 4		+
Mitogen-activated protein kinase kinase kinase kinase 5	+	
phosphoinositides		
Phosphatidylinositol 4-kinase type 2 beta	+	
Phosphatidylinositol binding clathrin assembly protein	+	
Phosphatidylinositol transfer protein, cytoplasmic 1	+	
Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III		+
Phosphatidylinositol-4-phosphate 5-kinase, type 1 beta		+
Phosphatidylinositol 3-kinase, C2 domain containing, alpha polypeptide		+
Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	+	
CONTROL OF GENE TRANSCRIPTION		
Mef2A	+	+
Pbx/knotted 1 homeobox	+	+
Nuclear receptor interacting protein 2	+	+
Deoxynucleotidyltransferase, terminal	+	+
BTB (POZ) domain containing 14A	+	+
MISCELLANOUS		
DIX domain containing 1	+	+
Sarcolemma associated protein	+	+
Sec63	+	+
Dihydrolipoamide S-acetyltransferase	+	+
Poly (ADP-ribose) polymerase family, member 6	+	+
Low density lipoprotein-related protein 1B	+	+

Fig. 4. VTA-specific deletion of BDNF and chronic administration of fluoxetine reverse the effects of social defeat stress on gene expression in the NAc. Gene expression in the NAc was evaluated using gene profiling (see SOM for a detailed experimental protocol and complete gene lists). **(A)** Hierarchical clusters of genes significantly up-regulated in the NAc of AAV-GFP–injected mice after 10 days of social defeat stress and how they are regulated by defeat across other experimental conditions. The results show that virtually all of these genes that were up-regulated 24 hours after defeat stress show the opposite regulation upon local deletion of BDNF from the VTA. Similarly, a large subset of the stress-regulated genes remain up-regulated after 4 weeks of treatment with vehicle, and most of these persistent changes are reversed by chronic treatment with fluoxetine. The intensity and direction of gene regulation are represented with a heat map (red, up-regulated; green, down-regulated). **(B)** Examples of genes significantly up-regulated ($+P < 0.05$) after intra-VTA BDNF knockdown or fluoxetine treatment in control mice. See tables S1 and S2 for detailed gene lists.

though this is in contrast to the reported effects of stress and antidepressants on BDNF signaling in the hippocampus (*10*), several manipulations that sensitize the dopaminergic system (*36*), such as social defeat, have been shown to increase dendritic branching in the NAc (*37*).

The demonstration that social stimuli become persistently aversive after repeated experiences of aggression in mice is one major finding of the present study. This behavioral phenomenon shares some similarities with persistent conditioned submissive responses that have been described previously in other rodent species (*22*).

Here, we took advantage of this observation to develop a murine model relevant to human psychiatric conditions such as depression, social phobia, and PTSD, in which social withdrawal is a common symptom. The observation that chronic but not acute treatments with antidepressant partly restore social approach behavior

in defeated mice further validates this model. Social cues stimulate the VTA-NAc pathway in mice, and this neural response becomes sensitized in defeated mice with social aversion. The second major finding of this study is the demonstration that intact BDNF function in the VTA is required for the development of this persistent social aversion. Our gene profiling study suggests that this process is mediated in large part by BDNF-regulated molecular pathways in the NAc and is counteracted by antidepressant drugs. The present results confirm our previous report that blockade of BDNF activity in the VTA-NAc pathway exerts an antidepressant-like activity in rodent models of stress (*38*). This profile is opposite to the antidepressant-like activity of BDNF reported in hippocampal studies (*39*) and suggests new directions for antidepressant drug discovery.

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Supporting Online Material

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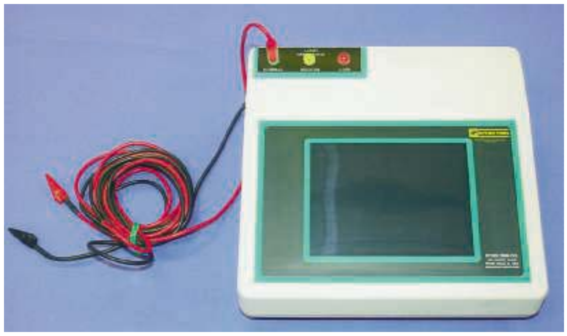

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Careers for Postdoctoral Scientists

The Ever-Aging Postdoc

Postdoctoral fellowships for many life scientists have become marathons that delay their entry to their first "real" jobs by some years. To counter the problem, potential employers in government, industry, and universities have started to set term limits to the postdoctoral experience. BY PETER GWYNNE

Employers in industry, government, and academe increasingly require the postdoctoral fellows they recruit to have a broad base of understanding of their fields. In many cases, postdocs can achieve that base only by devoting more time to their initial fellowship or by taking more than one postdoctoral stint. As a result, increasing numbers of postdocs now reach their late 30s before they take their first permanent job. "When I travel around, I'm shocked by the number of 40-year-old and even older assistant professors," says Michael Stryker, former chair of the physiology department at the University of California, San Francisco (UCSF). "It's clear in a lot of places that the period of postdoctoral training has become very prolonged."

Few statistics exist on the ages of postdocs when they gain their first employment. However, says Alyson Reed, executive director of the National Postdoctoral Association (NPA), "The average length of a fellowship seems to be in the neighborhood of four or five years." Typically, Stryker adds, "the people we hire for faculty positions have had about three years postdoctoral experience. But it ranges from zero in two cases to up to five years in the longest one I can think of. The five-year ones are often more than one postdoc."

Whatever the exact average, it represents an increase over the roughly three years that today's full professors took for their fellowships. Of course, science has become more difficult and complicated since then. "But it's not clear that the complexity of science should make a postdoc longer, because the tools have improved," says David Scheinberg, head of the molecular pharmacology and chemistry program at Memorial Sloan-Kettering Cancer Center.

THOMAS GINGERAS

Setting Term Limits

Supervisors of postdocs are recognizing the problem of excessively long postdoctoral training, and taking measures to minimize it. "There is definitely a trend toward setting time limits for postdoctoral appointments," Reed says. "Institutions that have been in the forefront of developing procedures to enhance the postdoctoral training experience are indeed aware of a holding pattern associated with some postdoctoral training programs. The National Institutes of Health has essentially set a term limit of five years for its training fellowships. And at least 22 of 74 academic institutions that have published data have their own term limits."

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National Postdoctoral Association
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University of California, San Francisco Physiology Department
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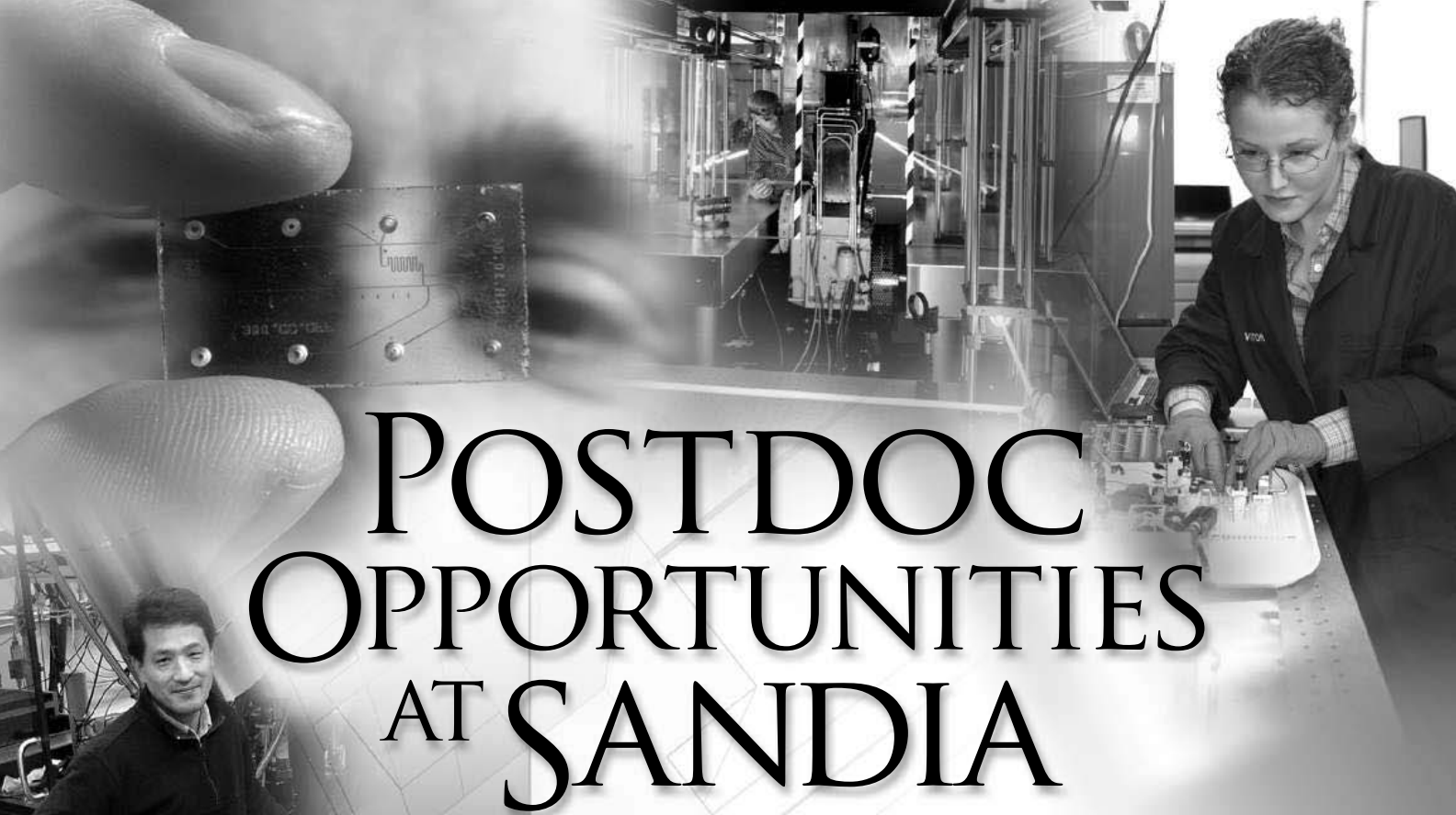
Such limits offer benefits to postdocs and potential employers. After spending three or four years on their research, postdocs may fear that they won't be employable. "If you wait too long, you begin to wonder whether your fellowship is an impediment to hiring," Scheinberg points out. Stryker agrees. "You can only stay in training positions for so long until you get a formal consideration of career prospects," he says.

That factor applies particularly to companies that seek to hire postdocs. "There are certainly many more opportunities in biotechnology and pharmaceuticals that are becoming interesting to the best and brightest postdocs, particularly in translation areas such as experimental therapeutics," says Scheinberg. "Most postdocs say: 'I need to do a second postdoc to get more published papers, or to stay another year in this postdoc so that I can get a better paper,'" Reed adds. "But if industrial recruiters looking at resumes see someone who's been a postdoc for more than five years, they begin to wonder what's wrong."

Significantly, postdoctoral fellowships in industry almost always take shorter times to complete than those in academe. CONTINUED »



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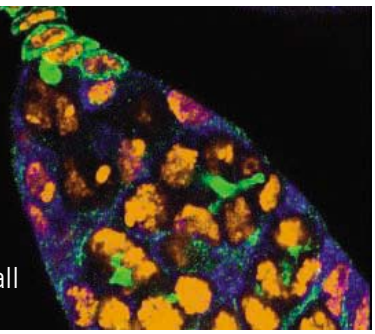

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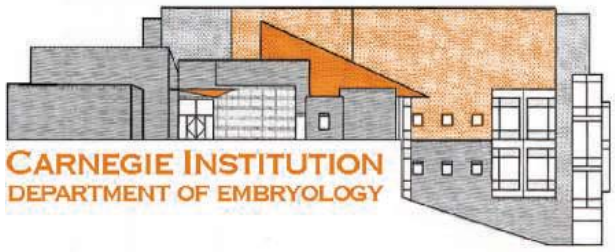

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Alex Bortvin,
Analysis of genetic and epigenetic control of mouse embryogenesis and germ cell development.

Identification and characterization of genes with key roles in embryonic and germ cell development in mice.

Donald Brown,
The control of gene expression in development.

Purified eukaryotic ribosomal RNA and 5S RNA genes from genomic DNA and analyzed their structure including the discovery of pseudogenes, spacer regions, internal promoters, and transcription termination sequences and amplification. Currently studying thyroid hormone control of gene expression in amphibian metamorphosis.

Chen-Ming Fan,
Molecular patterning and embryonic induction during mouse early development.

Identified tissue inductions that govern mammalian somite development and signaling pathways that controls these inductive events. Development and physiology of the neuroendocrine hypothalamus.

Steven A. Farber,
Biochemistry and genetics of modifiers of lipid metabolism during zebrafish development.

The accessibility and transparency of zebrafish embryos is exploited to study vertebrate physiology. These efforts primarily utilize fluorescent optical reporters to visualize lipid uptake and processing in vivo.

Joseph Gall,
Nuclear structure and function.

Described the structure of giant lampbrush chromosomes and ribosomal DNA amplification in amphibian oocytes. Developed the technique of in situ hybridization. Identified specific DNA sequences in heterochromatin and chromosomal telomeres. Currently studying the role of the Cajal body in transcription and RNA processing.

Marnie Halpern,
Zebrafish neural development.

Devised new approaches for mutational screening in the zebrafish model. Made inroads into correct development of the central nervous system and asymmetry of the vertebrate brain.

Douglas E. Koshland,
Structure, integrity and evolution of chromosomes.

Helped elucidate the molecular mechanism of higher order chromosome folding and its impact on chromosome segregation, recombination, repair and cell cycle transitions.

Yixian Zheng,
Mitosis and genome evolution.

Discovered new pathways that coordinate with the cell cycle machinery to regulate mitosis. Biochemically characterized gamma tubulin ring complex, a key protein complex required for microtubule nucleation and spindle assembly. Initiated a collaborative effort with Doug Koshland to study genome evolution in hybrid yeasts.

Careers for Postdoctoral Scientists

"Postdoctoral training periods are determined by the time required to complete both the training agreed to by the mentor and postdoctoral student and the research project that is taking place," says Thomas Gingeras, vice president of biological sciences at Affymetrix. "Experience points to at least a two-year training period." Scheinberg emphasizes the importance in academia of ongoing evaluation and communication between fellow and mentor about career goals and pathway opportunities.

ALYSON REED

Positions for Creative People

Employers, meanwhile, risk losing some of the ingenuity of young scientists if they wait too long to hire them. "I regard it as a necessity for us to be able to offer positions to people at the most creative times of their lives, which is shortly after their Ph.D.s," Stryker says. "The consensus for our search committees is that if someone has done Ph.D. research at the highest level in one lab and has done the same in postdoctoral work in another, they know that a lot of the credit must go to the young person no matter how excellent the reputation of his or her principal investigator. That's the time of life you want to have that person starting as an assistant professor." Scheinberg and his colleagues at Sloan-Kettering take a similar approach. "The critical factor in hiring postdocs for faculty positions," he says, "is the discoveries they have made."

Observers see several reasons for the extended time that today's postdocs take to complete their fellowships in life science. In a recent paper, Jennifer Ma of the TIAA-CREF Institute and Paula Stephan of Georgia State University attribute it to the increasing proportion of Ph.D.s being awarded in the life sciences and to adverse job market conditions that life scientists experience during their fellowships. Reed amplifies the latter point. "Where the job market is stronger, you see shorter postdocs or none at all," she says. "In disciplines where there's a glut, you see the holding pattern more for postdocs. That's particularly the case for postdocs looking for tenure-track, jewel-in-the-crown jobs."

Universities are also setting out to save money – and coincidentally reducing their recruitment of postdocs – by refusing to replace retiring professors in the traditional way. "They fill retired tenure-track positions with adjuncts and other nontenured individuals," Reed explains. "Postdocs shouldn't assume that every time a professor retires, it will be an opening for a tenure-track position."

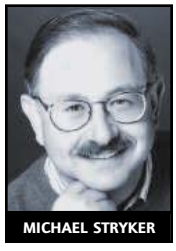
Grants and Other Concerns

Concern about gaining research grants also plays a role in dissuading some fellows from applying for jobs at early stages in their postdoctoral work. "There is a worry among some postdocs that, given the current contraction of federal funding, they will be launched into careers without the ability to rapidly gain external funding," Sloan-Kettering's Scheinberg says. "There is some trepidation about getting out too fast without a substantial body or work." That fact applies particularly to postdocs in university departments outside the top tier. "At the best institutions we never consider even for a millisecond whether anyone has independent grant support in making an offer for them to become an assistant professor; we are confident that anyone we hire will eventually get support," UCSF's Stryker explains. "But in less competitive institutions, it's a tremendous advantage to a young person to have obtained independent research support. For the institution hiring them, they are a lot cheaper."

DAVID SCHEINBERG

In certain cases, postdocs need extra time to complete their fellowships. "Some of the laboratories most in demand get very talented scientists who apply too late in their graduate careers to get in immediately. So they do something else in the interim," UCSF's Stryker says. "Some of the most brilliant ones have done this because they didn't get their lives organized enough as graduate students to find a place in the lab they most wanted. They come to us after another brief postdoc."

Another exceptional group needs more basic training. "In some of the most integrated areas of biology, notably systems biology as it evolves, there are people who come to life science laboratories with backgrounds in physics or mathematics or other hard sciences," Stryker continues. "These people generally take longer for their postdoctoral training; they get the training that a biology Ph.D. would need in addition to the postdoc. For them, the average length of fellowship is probably five years. Some have had training periods as long as seven years."



MICHAEL STRYKER

Approaches of the Elite

Elite academic institutions have largely led the way in methods of reducing the time taken for postdoctoral research. "UCSF, like many institutions, has rigidly distinguished between research staff and training positions – scientists who have all the perks of employees versus students," Stryker explains. "That has brought home to the faculty that faculty members have an educational responsibility to their postdoctoral fellows."

But the change of title from postdoc to research staffer can't disguise the fact that faculty jobs for postdocs are rare and highly competitive. "If you're an academic research associate, it may sound like you have a fulfilling position in academia," Reed says. "But perhaps only 20 percent of those scientists are potential tenure-track faculty in biomedicine."

Stryker points out the basic fact that supervisors and their postdocs must bear in mind. "There's educational value in some period of years, such as three years – or five years for physicists and mathematicians – of being in a training position," he says. "But at some point they're not training any more; they're in career paths as independent scientists."

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

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Genentech's research organization features world-renowned scientists who are some of the most prolific in their fields and in the industry. Genentech researchers have consistently published at a rate of 150+ papers per year and have secured more than 4,600 patents worldwide (with 5,000 more pending). Genentech's research organization combines the best of the academic and corporate worlds, allowing researchers not only to pursue important scientific questions but also to watch an idea move from the laboratory into development and out into the clinic. We are proud of our long history of groundbreaking science leading to first-in-class therapies, and we hope you'll consider joining us as we continue the tradition.

Postdoctoral Opportunities

Postdoctoral Research Fellow – Req.# 1000009244
A postdoctoral opportunity is available in the Department of Bioinformatics under the supervision of Thomas Wu, M.D., Ph.D., to develop and apply novel computational techniques in the areas of genomics or microarray analysis of gene expression or DNA copy number.

Postdoctoral Research Fellow – Req.# 1000009613
A postdoctoral opportunity is available in the Department of Protein Engineering in the NMR facility to develop and apply techniques for studying the solution structures of proteins and protein/protein or protein/ligand complexes of therapeutic interest.

Postdoctoral Research Fellow – Req.# 1000009624
A postdoctoral opportunity is available in the Department of Immunology to study lymphocyte signaling and function in normal and autoimmunity. The successful candidate will have a demonstrated graduate studies record of innovative scientific accomplishment and motivation to study underlying mechanisms of in vivo.

Postdoctoral Research Fellow – Req.# 1000010229
A postdoctoral opportunity is available in the Department of Immunology for a highly motivated researcher to study the role of macrophages in innate immunity.

Postdoctoral Research Fellow – Req.# 1000008860
A postdoctoral opportunity is available in the Department of Biomedical Imaging to study structural and physiological responses to therapy. The areas of potential projects include MR cell trafficking, targeted contrast agents and the study of angiogenesis by MRI.

Postdoctoral Research Fellow – Req.# 1000010722
A postdoctoral opportunity is available in the Department of Molecular Biology to investigate novel signaling pathways involved in cancer and/or membrane trafficking, as well as establish a cell-based fluorescent protein screen for genes involved in formation of or transport within primary cilia.

To learn more about these opportunities, please visit www.gene.com/careers. Please use "Ad - Science Mag" when a "source" is requested. Genentech is an equal opportunity employer.

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POSITIONS OPEN

POSTDOCTORAL TRAINING FOR PHYSICIANS AND VETERINARIANS IN TRANSLATIONAL BIODEFENSE/EMERGING INFECTIOUS DISEASE RESEARCH. Positions supported by the NIH/National Institute of Allergy and Infectious Disease Region VIII Regional Center for Excellence in Biodefense Grant are available at the University of Colorado at Denver and Health Sciences Center (UCDHSC) for advanced postdoctoral training leading to careers in biodefense-related clinical/translational research. Candidates should have M.D., M.D./Ph.D., D.V.M., or D.V.M./Ph.D. degrees, demonstrated competency and productivity in research, and strong interest in obtaining training in translational research involving Select Agents and/or emerging infectious diseases. *Applicants pursuing biodefense research must be U.S. citizens or permanent residents of the United States,* and appointment is contingent on receiving federal authorization to work with Select Agents at UCDHSC. Positions are available immediately, starting dates are flexible, and successful candidates are eligible for support for up to two years from this funding source. The research facilities and the training environment at UCDHSC are excellent. Compensation is determined by NIH policies for postdoctoral training. To apply send curriculum vitae and bibliography, names of three professional references with contact information, and a cover letter describing both past research experience and future career goals in biodefense/emerging infectious disease-related research. Send application materials to: **Randall K. Holmes, M.D., Ph.D., UCDHSC Department of Microbiology, Mail Stop 8333, P.O. Box 6511, Aurora, CO 80045, or e-mail: randall.holmes@uchsc.edu.** *The University of Colorado at Denver and Health Sciences Center is committed to Equal Opportunity and Affirmative Action.*

POSTDOCTORAL POSITION
Available Immediately

NIH-funded Postdoctoral Position to study mechanisms underlying lower urinary tract dysfunction following spinal cord injury or bladder inflammation. The roles of neurotrophic factors in injury/dysfunction/development are investigated with a multidisciplinary approach that includes: anatomical tracing, immunostaining, electrophysiology, biochemical, and molecular assays. Previous experience in several of these approaches is required. Applicants must have strong motivation and excellent communication skills. Funding is available for at least two years.

Applicants should send curriculum vitae and bibliography, names and addresses of three references to: **Margaret A. Vizzard, Ph.D.** and **Gary M. Mawe, Ph.D., University of Vermont, Department of Anatomy and Neurobiology, D-411 Given, Burlington, VT 05405 U.S.A. Telephone: 802-656-3209; e-mail: margaret.vizzard@uvm.edu** or **gary.mawe@uvm.edu.**

POSTDOCTORAL POSITIONS. Two Postdoctoral Positions are available for the chemical biology and biophysical studies of lipid signaling and membrane trafficking. Our interdisciplinary research involves specific labeling and real-time quantitative measurements of cellular lipids and proteins by multiphoton microscopy in addition to in vitro biophysical studies. This research group provides a highly challenging and supportive environment with state-of-the-art facilities. Energetic candidates with a strong background in biochemistry, cell biology, or microscopy are encouraged to apply. Please send curriculum vitae and names of three references to: **Wonhwa Cho, Department of Chemistry (M/C 111), University of Illinois at Chicago, 845 W. Taylor Street, Chicago, IL 60607. E-mail: wcho@uic.edu. Website: http://brahms.chem.uic.edu.**

POSTDOCTORAL POSITION on NIH funded project to develop novel medical devices related to blood purification and treatment. Seeking Ph.D. in biomedical engineering with experience in fluid mechanics and mass transfer to perform modeling and related experiments. **E-mail: johnsonnm2@upmc.edu,** or visit **website: http://www.mirm.pitt.edu/medicaldevices/.**

POSITIONS OPEN

CALIFORNIA INSTITUTE OF TECHNOLOGY
Broad Fellows Program in Brain Circuitry

The California Institute of Technology is looking for a few outstanding Scientists from any relevant backgrounds to study how networks of neurons give rise to perception, memory, emotion, and behavior. We encourage applications from individuals employing genetic manipulations in relevant animal model systems, electrophysiological recordings, functional imaging and computational analyses and related tools. Broad Fellows are independent researchers who have recently received their Ph.D. They will receive internal funding for a group of up to three people. The initial appointment is for three years, with the possibility of renewal for two more years. Excellent salary and benefits. Applications should include curriculum vitae, a statement of research plan, and three letters of recommendation. This material should be sent to: **Heather Hein, Broad Fellows Search Committee, California Institute of Technology, MC 216-76, 1200 E. California Boulevard, Pasadena, CA 91125,** or by **e-mail: heather@klab.caltech.edu.**

Caltech is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

RESEARCH SCIENTIST

Stony Brook University's Department of Pediatrics, Division of Neonatology, has an immediate opening for a Research Scientist to study the mechanism and regulation of lung surfactant secretion. The focus will be the regulation of membrane fusion in exocytosis, see *Biochimica et Biophysica Acta* **1734:** 152-168, 2005. Required: Ph.D. in biochemistry or molecular biology; three or more years of postdoctoral experience in a related research discipline, including one year of experience in expression, purification, and characterization of recombinant proteins as evidenced by publication(s), and one year in experimentation with cultured mammalian cells. Preferred: experience with cell culture, characterization, and experimentation with lung cells. Send resume to: **Avinash C. Jerath, Ph.D., Research Professor, Department of Pediatrics, HSC T11 060, Stony Brook University, Stony Brook, NY 11794-8111. Fax: 631-444-8968.** To apply online visit our **website: http://www.stonybrook.edu/cjo.** *Affirmative Action/Equal Opportunity Employer.*

NIH TRAINING GRANT
POSTDOCTORAL FELLOW

A Postdoctoral Fellow position is available in the Rheumatology Division of the Medical University of South Carolina. There are ten senior mentors who serve on the training grant centered on inflammatory and fibrotic diseases. The Directors of these laboratories are involved in translational research in lupus and scleroderma. Pharmacologic, immunologic, molecular biologic, and genetic approaches are being taken to study these complex diseases. *To qualify for this position the individual must be a U.S. citizen or permanent U.S. resident. J1 or H1 visas are not acceptable.* If interested please send your curriculum vitae and a cover letter to: **Dr. Gary Gilkeson** via **e-mail: gilkeson@musc.edu.**

POSTDOCTORAL POSITION available immediately to study the structure of the Bacillus anthracis protective antigen (**website: http://mrce.wustl.edu/Bann.html**) using fluorine nuclear magnetic resonance (NMR). Candidates should have experience in NMR and experience in molecular biology and biochemical techniques (mutagenesis, protein purification) is desirable. Interested applicants should send curriculum vitae and three letters of recommendation to: **Dr. Jim Bann, Department of Chemistry, Wichita State University, 1845 Fairmount, Wichita, KS 67260-0051. E-mail: jim.bann@wichita.edu.** *Wichita State University is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

RESEARCH/TEACHING POSTDOCTORAL POSITION, PLANT GENETICS. Project involves mapping of genes regulating the HMW glutenin and starch biosynthesis enzyme genes in wheat. Experience in plant genetics, basic molecular biology techniques including real time RT-PCR, and database utilization desirable. Candidate will carry out research, mentor undergraduate students in research, and have opportunity to gain teaching experience. Excellent training opportunity for someone seeking career at a primarily undergraduate institution. One year position, with possibility of extension to three years, starting June 2006. Ph.D. required. See **website: http://www.augie.edu/dept/biology/Web/faculty/Wanous/Wanous.html.** Submit application and curriculum vitae to **e-mail: mike.wanous@augie.edu.** Also have three letters of recommendation sent to: **Dr. Michael Wanous, Department of Biology, Augustana College, 2001 S. Summit Avenue, Sioux Falls, SD 57197 U.S.A. Telephone: 605-274-4712.** Review of applications will begin March 10, 2006. *Augustana College is an Equal Opportunity/Affirmative Action/Title IX Employer. Qualified Women and Minority Applicants Encouraged to Apply.*

NORTHERN KENTUCKY UNIVERSITY

POSTDOCTORAL POSITION is available to characterize members of the P5 subfamily of P-type transport ATPases in mice. Research involves the expression, localization, and determination of the ion specificity and cellular/physiological function of the transporters. Applicants should have a Ph.D. with a strong background in molecular biology/biochemistry. Opportunities to teach undergraduate laboratory or lecture courses are also available but not required. Salary is commensurate with experience but competitive. Please submit curriculum vitae, summary of research experience, and three references to:

Patrick J. Schultheis, Ph.D.
Department of Biological Sciences
Northern Kentucky University, Nunn Drive
Highland Heights, KY 41099
E-mail: schultheisp@nku.edu

For best consideration, please submit materials by April 1, 2006. *Northern Kentucky University is an Affirmative Action/Equal Opportunity Employer and actively seeks applications from minorities and women.*

POSTDOCTORAL POSITION

A Postdoctoral Fellowship position is available in the laboratory of **Dr. Zuhair Ballas**, at the University of Iowa (*Alcohol* **33:**175, 2004; *Blood* **105:** 682, 2005; *DNA Cell Biol.* **22:** 621, 2003*; J. Clin. Investig.* **109:**1501, 2002; *Immunol.* **167:** 4878, 2001). Applicants must have a Ph.D. and should have experience in immunology, cell biology, or signaling in both human and murine lymphocytes. The laboratory currently focuses on the role of NK cells in health and disease. Applications, including curriculum vitae and bibliography, summary of past accomplishments, and names of three references should be sent to: **Zuhair K. Ballas, M.D., Division of Allergy/Immunology, Department of Internal Medicine, 200 Hawkins Drive, University of Iowa, Iowa City, IA 52242. Telephone: 319-356-3697; fax: 319-356-8280; e-mail: ballasz@uiowa.edu.**

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

POSTDOCTORAL FELLOWSHIP. The research concentrates on models of chronic oxidative deficits that produce selective neurodegeneration, oxidative stress, cholinergic deficits, and memory loss. In vivo approaches (especially immunocytochemistry but also behavioral, molecular biological, biochemical approaches, in situ hybridization, confocal microscopy and electron microscopy) and in vitro strategies (primary cultures of endothelial cells, microglia, neurons, astrocytes and fibroblasts) are utilized. Contact: **Dr. Gary E. Gibson, Cornell Medical College,** at **Burke Medical Research Institute, White Plains, NY 10605. Telephone: 914-597-2291; fax: 914-597-2757; e-mail: ggibson@med.cornell.edu.**

Post-doctoral Positions Available in the Vascular Biology Center

The mission of the Medical College of Georgia (MCG) is to improve health and reduce the burden of illness in society by becoming one of the nation's premier health sciences Universities. MCG is located in the growing and thriving city of Augusta, recently ranked the second most favorable place to live in Georgia.

The city offers a wide array of cultural and recreational activities. Outdoor activities such as water-skiing, swimming, boating and camping abound. In addition to being a magnet for world-class researchers, Augusta offers affordable living, high-quality schooling, a rich culture, and is within an easy three-hour drive of Atlanta, the Atlantic Ocean, and the mountains.

Post-doctoral (Ph.D. required) positions are currently available within the Vascular Biology Center (**www.mcg.edu/centers/VBC**) to join an NIH-funded multi-disciplinary team investigating the effects of biomechanical forces and oxidative stress on cell signaling in blood vessels. For prompt consideration, please send a CV with three references to: **Dr. Stephen M. Black (MCG-Black@att.net).**

The Medical College of Georgia is an Affirmative Action, Equal Opportunity Employer.

POSTDOCTORAL POSITIONS IN SWEDEN

The Swedish Research Council's programme of postdoctoral positions enables researchers with Swedish or non-Swedish doctorate degrees (PhD or equivalent) to work at Swedish higher education institutions or research establishments for up to two years.

LAST APPLICATION DATE

Humanities and Social Sciences	April 19
Educational Science	April 19
Natural and Engineering Sciences	April 25
Medicine	April 27

FURTHER INFORMATION AT:

www.vr.se

Application documents will be posted on the website at the end of February.

Vetenskapsrådet

The Swedish Research Council is a government agency funding basic research of the highest scientific quality in all disciplines. The Swedish Research Council has a national responsibility to support and develop basic research and promote research innovation and research communication. The goal is for Sweden to be a leading nation in scientific research.

POST-DOCTORAL RESEARCH ASSOCIATE POSITION in insect functional genetics/genomics through the School of Life Sciences at Arizona State University. Candidate must have earned a doctoral degree in molecular/functional genetics, genomics or a related field prior to appointment and must not currently hold a permanent faculty position; those with a demonstrated productive and innovative research program will be given preference. Backgrounds in studies of insulin/insulin-like signaling and laboratory work with model organisms are desired but not essential. Position will entail studies of gene regulatory networks underlying social behavior, and interlinkage between social behavior, reproductive physiology and aging using the honey bee (*Apis mellifera*) as model. Duties will include gene expression profiling (microarray/suppression subtractive hybridization/real time-RT PCR) paired with protein expression analyses (immunoprecipitation, Western), gene knockdown (RNA interference), and studies of social behavioral phenotypes. The position could begin as soon as May 1, 2006.

Send cover letter summarizing your qualifications and interests, curriculum vitae, representative reprints and names and emails of three professional references to: **Dr. Gro V. Amdam, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501**. Email submissions are acceptable (**Gro.Amdam@asu.edu**). File review will begin **March 15, 2006**; if not filled weekly thereafter until search is closed. A background check is required for employment.

AA/EOE

Department of Health and Human Services
National Institutes of Health
National Institute on Aging

The National Institute on Aging, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is recruiting for **four post-doctoral fellows** in the Laboratory of Genetics, Intramural Research Program (IRP):

1) with a background in cell based screens or imaging studies to work in the Image Informatics and Computational Biology Unit (IICBU), for high-throughput automated visual screening of RNAi libraries. The interdisciplinary group has developed image classification algorithms based on machine learning techniques, and we would like to apply these to the systematic reconstruction of genetic pathways. For additional information on this research, please go to: **(http://www.grc.nia.nih.gov/branches/lg/iicbu/iicbu.htm)**. Applicants should send the curriculum vitae, via email to Dr. Ilya Goldberg at, **goldbergil@grc.nia.nih.gov**.

2) with a background in biochemistry to work in the Transcription Regulation and Remodeling Section (TRRS), on purification of multi-protein complexes and analysis of their structures and functions **(http://www.grc.nia.nih.gov/branches/lg/trru/trru.htm)**. Projects include studies of chromatin-remodeling mechanisms (*G&D 19:1662-7*), DNA damage response, and human genomic instability diseases (*Nat. Genet. 35:165-170; 37: 958-63*). Applicants should send the curriculum vitae, via email to Dr. Weidong Wang, **wangw@grc.nia.nih.gov**

3) with a background in mouse development to work in the Developmental Genomics and Aging Section, to conduct the study of preimplantation mouse development (*Dev. Cell 6: 117-131, 2004*) and embryonic stem cells (*PLoS Biol. 1: 410-419, 2003*). The work utilizes embryogenomics approaches (*Trends Biotechnol. 19: 511-518, 2001*) and focuses on the identification and characterization of genes that are critical for the maintenance of pluripotency and/or for early commitment to different cell lineages. Applicants should send the curriculum vitae via email to Dr. Minoru Ko, **kom@grc.nia.nih.gov**.

4) with a background in molecular genetics to work in the Human Genetics Section, on the determination of skin appendage formation in vitro, based on signaling pathways operating with the EDA (ectodysplasin) TNF-ligand (*Hum. Molec. Genet. 11:1763-1773; Hum. Molec. Genet. 12: 2931-2940*). The aims include the understanding of how hair follicles form, as a model system for both development and possible regeneration. Approaches include histology, keratinocyte cell differentiation, and immunocytochemistry, as well as a range of genomic and physiological techniques. Applicants should send the curriculum vitae, via email to Dr. David Schlessinger, **schlessingerd@grc.nia.nih.gov.**

The successful individuals will possess an M.D. or Ph.D. degree in biochemistry, molecular genetics or a related field, with no more than five years of Post Doctoral research experience. Salary is commensurate with research experience and accomplishments.

DHHS and NIH are Equal Opportunity Employers

POSITIONS OPEN

POSTDOCTORAL POSITIONS
Molecular Cell Biology of Diabetic Complications

As reviewed in *Nature* **414:** 813, 2001, our laboratory focuses on the mechanisms by which hyperglycemia causes vascular damage. We are currently investigating (a) the molecular basis for "metabolic imprinting," (b) the genetic basis for familiar clustering of susceptibility to hyperglycemic damage, (c) endothelial progenitor cell dysfunction and impaired vasculogenesis in diabetes, and (d) identification of novel therapeutic strategies for preventing metabolite-induced vascular damage. Candidates should have a strong foundation in molecular and cell biology. (See **Yao, D. et al.,** *Cell* **124:** 275286, 2006.) Please send curriculum vitae and names and contact information of three references to: **Dr. M. Brownlee, Diabetes Research Center, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx NY 10461; e-mail: brownlee@aecom.yu.edu.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION available to investigate the molecular biology of Epstein-Barr virus mediated transformation of B-cells into immortalized cell lines. For more information, see our **website: http://www.uthscsa.edu/micro/.** This position is supported by NIH/National Cancer Institute grant award and is located in the Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio (UTHSCSA). Applicants should have a doctoral degree in microbiology, molecular or cell biology, biochemistry, or related biomedical science. Salary will be commensurate with experience. Applications sent by mail, fax, or e-mail should include curriculum vitae and contact information for three referees. Address these to: **Dr. Kenneth Izumi, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas, 78229-3901. E-mail: izumi@uthscsa.edu, fax: 210-567-6612.** All Postdoctoral Fellow appointments are designated as security sensitive positions. *UTHSCSA is an Equal Employment Opportunity-Affirmative Action Employer.*

POSTDOCTORATE FELLOW
Department of Anesthesiology
New York University
School of Medicine

We are inviting applications for a Postdoctoral Position to work on the molecular mechanism of Barth syndrome. Barth syndrome is a mitochondrial disease involving primarily heart and skeletal muscle. The successful applicant will use standard cell culture, molecular biology, and lipid analytical techniques to investigate the biological function of tafazzin. Experience in mitochondrial biogenesis research is desirable, but not a condition. The project is funded by the NIH for a period of four years with possible extension.

Please e-mail curriculum vitae and cover letter to **e-mail: janice.rothermel@med.nyu.edu,** or mail to: **Janice Rothermel, Department of Anesthesiology, 550 First Avenue, IRM607A, New York, NY 10016.**

POSTDOCTORAL POSITION available for brain cancer research to evaluate the therapeutic potential of genes mutated in glioblastomas. For this NIH-funded project, strong experience in molecular biology is required. Send curriculum vitae to: **Dr. Greg Riggins, Department of Neurosurgery, 1550 Orleans Street, Room 2.57 Baltimore, MD 21231. E-mail: griggin1@jhmi.edu.** *Johns Hopkins is an Affirmative Action/Equal Opportunity Employer.*

Full-time **POSTDOCTORAL RESEARCH FELLOWSHIP POSITION** available at **Brigham & Women's Hospital, Harvard Medical School.** Focus of laboratory centers on developmental biology of hematopoiesis using zebrafish as model system. Candidates with doctoral degrees, *U.S. citizenship or permanent residency,* and background in biological sciences are encouraged to apply. Please send enquiries and curriculum vitae to: **Barry Paw (e-mail: bpaw@rics.bwh.harvard.edu).**

The Host Microbe Systems Research Theme in the Institute for Genomic Biology (**website: http://www.igb.uiuc.edu**) at the **University of Illinois** currently has an opening for a **POSTDOCTORAL FELLOW IN MICROBIAL ECOLOGY.** The Fellow will be responsible for developing DNA isolation and 16S rRNA and other ribotyping and metagenomic library construction techniques for surveying microbial content of the vagina. Additional responsibilities will include the development of molecular biology and genomic techniques to examine vaginal contents and performing analyses using bioinformatics and other computational and analytical methods. The ideal candidate will have a strong background in microbiology, biochemistry, chemistry, or a related field with experience and expertise in molecular microbial ecology and bioinformatics. Closing date for the position is April 1, 2006. Please submit your curriculum vitae, and names of three references to: **Professor Brenda Wilson, e-mail: bawilson@life.uiuc.edu.**

SCIENCE WRITER

The Rockefeller University seeks a Science Writer to join the Rockefeller University Press. Responsibilities include selecting and writing items for news coverage in the *Journal of Cell Biology, Journal of Experimental Medicine*, and *Journal of General Physiology*, writing press releases, traveling to international meetings, commissioning and editing commentaries, and coordinating production of news pages with production office.

Ph.D. degree in biological sciences required; journalistic writing experience preferred. We offer an excellent benefits package and competitive salary. For immediate consideration, please send resume, clips, and salary requirements to: **The Rockefeller University, 1230 York Avenue, Box 125, New York, NY 10021. Fax: 212-327-7079; e-mail: recruit@rockefeller.edu.** *An Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION
Microbial Pathogenesis

Available immediately to study the molecular mechanism of type III secretion in Yersinia enterocolitica using microbial genetics, biochemistry, and electron microscopy. Diverse projects are available but the focus is the structure/function and the cell contact-dependant regulation of type III secretion. Applicants with experience in biochemistry, genetics and/or cell biology should send curriculum vitae, contact information of three references, and a brief description of their interests to: **Dr. Egbert Hoiczyk, Department of Molecular Microbiology and Immunology, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: ehoiczyk@jhsph.edu.** For more information visit **website: http://faculty.jhsph.edu/?F=Egbert&L=Hoiczyk.**

POSTDOCTORAL FELLOW

A Postdoctoral Fellowship is available immediately to study molecular mechanisms of cytomegalovirus latency and reactivation. Candidates must have a Ph.D. and significant background in molecular biology. Ability to communicate well in English is essential. Please submit your curriculum vitae and names of three references to: **Dr. Mary Hummel, Northwestern University Feinberg School of Medicine, Transplant Laboratory, Department of Surgery, Mail Code T231, 303 E. Chicago Avenue, Chicago, IL 60611. E-mail: m-hummel@northwestern.edu.**

PH.D. STUDENTSHIPS AND POSTDOCTORAL FELLOWSHIPS to study meiotic oocyte maturation, using both frogs and mice as model organisms. We are equipped with in-laboratory facilities to handle microinjection and time lapse confocal imaging of both frog and mouse oocytes. Please send curriculum vitae and a brief statement of accomplishment/experience, along with names of three references to: **Dr. X. Johné Liu, Ottawa Health Research Institute, 725 Parkdale Avenue, Ottawa, K1Y 4E9, Canada. E-mail: jliu@ohri.ca. Website: http://www.ohri.ca/profiles/liu.asp.**

POSTDOCTORAL TRAINING, CANCER RESEARCH. The Department of Biochemistry and Molecular Biology in the Johns Hopkins Bloomberg School of Public Health is pleased to announce a new National Cancer Institute funded postdoctoral training program in cancer research. This program provides training in basic biochemical, biophysical, and molecular biological approaches that can be applied to critical problems in cancer biology. Trainees will carry out a traditional laboratory- based research project and in addition will receive training and participate in activities that provide an introduction to current topics in the clinical aspects of the cancer problem and their relationships to public health. Applicants must have a Ph.D. or M.D. degree, a strong academic record, significant research experience in the biological, chemical, or physical sciences and *must be citizens or permanent residents of the United States*. Information about the program and instructions for applying may be found at **website: http://www.jhsph.edu/dept/BMB.**

POSTDOCTORAL POSITIONS available for rational design of anticoagulants. The projects involve molecular modeling, organic synthesis, and biochemical experiments. The candidate must hold a Ph.D. in any of these fields. Send cover letter along with curriculum vitae and any relevant information, and arrange for three letters of recommendation to be delivered to: **Professor Umesh R. Desai, Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, 800 East Leigh Street, Suite 221, Richmond, VA 23219.**

HARVARD MEDICAL SCHOOL

POSTDOCTORAL POSITION available to study the molecular and cellular mechanisms of neurodegenerative diseases and mental retardation. Recent Ph.D.s with strong background in molecular biology are encouraged to apply. Send curriculum vitae to: **Dr. Jie Shen (website: http://www.shenlab.net)** at **e-mail: jshen@rics.bwh.harvard.edu,** or **Raymond Kelleher** at **e-mail: kelleher@helix.mgh.harvard.edu.**

POSTDOCTORAL ASSOCIATE. An NIH-funded position is available in the structural studies of membrane proteins using X-ray crystallography. Specific focus is on multi-drug efflux pumps that recognize an enormous range of structurally dissimilar compounds from cells. Send curriculum vitae and names of three references to: **Dr. Edward Yu, Department of Physics and Astronomy, Iowa State University, Ames, IA 50011 U.S.A. E-mail: ewyu@iastate.edu.**

POSTDOCTORAL POSITION available immediately in the area of skeletal tissue engineering to study matrix formation in cell-responsive scaffolds seeded with stem cells. Applicants should hold a Ph.D. in cell biology or related field with strong background in stem cell isolation, culture, and characterization. Please e-mail curriculum vitae to: **Professor Esmaiel Jabbari, Chemical Engineering, University of South Carolina, e-mail: jabbari@engr.sc.edu.** *Equal Opportunity Employer.*

A **POSTDOCTORAL RESEARCH POSITION** is available at the **University of Florida** emphasizing kinetic and structural methods to investigate the mechanisms of fast enzymes such as carbonic anhydrase and superoxide dismutase. Please send curriculum vitae and the names and addresses of three references to: **Dr. David Silverman, e-mail: silvermn@college.med.ufl.edu.** *The University of Florida is an Equal Opportunity Employer.*

POSTDOCTORAL POSITION to study the human immune response to Chlamydia trachomatis and host-pathogen interaction. Training in immunology or microbial pathogenesis desirable. Please send curriculum vitae with references to: **Dr. Paula Kavathas, Section of Immunobiology Yale University. E-mail: paula.kavathas@yale.edu**

CANADIAN BLOOD SERVICES
SOCIÉTÉ CANADIENNE DU SANG

Postdoctoral Fellowships

Canadian Blood Services (CBS) is accepting applications for Postdoctoral Fellowships (PDF) to work with our affiliated Research and Development groups across Canada. CBS has active research programs within transfusion science emphasizing platelets, stem cells, plasma proteins, infectious disease, epidemiology and clinical transfusion practice. Applicants should have a Ph.D. or M.D. degree and a strong research background. This two-year award includes a salary and research allowance, and the possibility of a one-year renewal. Candidates must select and contact a CBS affiliated scientist to serve as the Postdoctoral Fellowship supervisor. CBS also supports a Graduate Fellowship Program and a Summer Internship Program.

Information, forms and a list of CBS affiliated scientists are available at **www.bloodservices.ca**, and from the R&D Office (**elaine.konecny@bloodservices.ca**), **Canadian Blood Services, Research and Development, 1800 Alta Vista Drive, Ottawa, Ontario, K1G 4J5, Canada**.

PDF Application deadline: July 5, 2006.

POSTDOCTORAL OPPORTUNITIES

The Wadsworth Center of the New York State Department of Health, with basic and applied research programs in the biomedical and environmental sciences, provides a unique and dynamic postdoctoral training experience. Enhancing this environment are state-of-the-art core facilities; broad-based graduate programs with the University at Albany, State University of New York; and new initiatives in bioinformatics, genomics, nanobiotechnology, and biodefense. Positions are available in the following areas:

- **Atmospheric Chemistry**
- **Biodefense**
- **Biomarkers/Nutrition**
- **Cancer Biology/Chemotherapy**
- **Carcinogenesis**
- **Cell Biology/Mitosis**
- **DNA Repair/NMR**
- **Drug Metabolism/Resistance**
- **Gene Expression/Regulation**
- **Immunology**
- **Infectious Disease**
- **Medical Entomology**
- **Microbial Genetics/Pathogenesis**
- **Mobile Genetic Elements**
- **Neuroscience/Disease**
- **Stem Cell Biology**
- **Structural Biology**
- **Toxicology/Neurotoxicology**

For additional information, go to:
www.wadsworth.org/educate/postdocs.htm
and to apply, contact:
Dr. Donal Murphy, Research Office,
Wadsworth Center, New York State Department of Health
P.O. Box 509, Albany, NY 12201-0509
murphy@wadsworth.org

Wadsworth Center
New York State Department of Health
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AA/EOE

UCI
University of California, Irvine

Postdoctoral Scholar Positions

Postdoctoral Scholar positions are immediately available to study genomic instability, mitotic checkpoint and chemical biology. Recent publications: DNA damage and checkpoint signaling (*PNAS* **98:**13102, *Nature* **405:**473, *Cancer Res.* **65:**1158, *JBC* **278:**8873) BRCA1 and 2 (*Mol. Cell* **6:**757, *Science* **297:**1837, *Science* **285:**747, *PNAS* **102:**9176) Hec1 (*MCB* **19:**5417) CTIP (*Nature* **406:**210, *MCB* **25:**3535). Annual salary ranges $31,668-$46,980 depending on research experience. Training and experience in molecular biology, cell biology, biochemistry preferred.

Prospective candidates should forward their CV and three references to:

Dr. Wen-Hwa Lee
University of California, Irvine
Department of Biological Chemistry
Irvine, CA 92697-1700
Fax: 949-824-2688
E-mail: whlee@uci.edu

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity.

National Exposure Research Laboratory Post-Doctoral Program

- The National Exposure Research Laboratory (NERL) of the United States Environmental Protection Agency is accepting applications beginning February 6 through April 7, 2006 for a number of federal, three-year post-doctoral research positions.
- Candidates will engage in research in areas such as environmental monitoring and characterization; computer modeling of the transport, transformation, and fate of pollutants in multiple media and at multiple scales; human and ecological exposure analysis; remote sensing applications; and landscape ecology.
- Specific research opportunities are posted on the NERL website at **http://www.epa.gov/nerl.**
- Post-doctoral positions will be in one or more of the following locations: Research Triangle Park, North Carolina; Cincinnati, Ohio; Las Vegas, Nevada; Athens, Georgia; or Washington DC metropolitan area.

FULL FEDERAL EMPLOYMENT BENEFITS:

- Salary range of $51,972 - $84,257
- Full three-year appointments
- Paid relocation to EPA duty location
- Federal health benefits, life insurance, and retirement program
- Flexible start date in 2006
- Vacation and sick leave
- Travel to professional and scientific meetings

APPLICATION PROCESS – Consult the NERL website at **http://www.epa.gov/nerl** for instructions on how to apply. Note – online applications are not accepted. Applicants must provide:

- Up-to-date Curriculum Vitae
- Letter of recommendation from your research advisor or comparable official
- Cover letter indicating: positions and locations of interest; your email address; U.S. Citizenship status, AND how you learned of this program
- DD-214, if claiming veteran's preference

Applicants must be United States citizens or permanent residents. Only in the absence of qualified U.S. citizens will permanent residents who are citizens of countries specified as exceptions to the appropriations act ban on paying non-U.S. citizens be considered.

Specific job information is posted on the NERL Internet site at **http://www.epa.gov/nerl**. EPA provides reasonable accommodations to applicants with disabilities. If you need a reasonable accommodation for any part of the application and hiring process, please notify the Agency. The decision on granting reasonable accommodation will be on a case-by-case basis.

The U.S. EPA is an Equal Opportunity Employer.

Joslin Diabetes Center is an internationally recognized diabetes treatment, research and education institution affiliated with Harvard Medical School and headquartered in Boston, MA. Joslin is a non-profit organization dedicated to finding a cure for diabetes and improving the lives of people with diabetes through its cutting-edge basic and clinical research, patient care programs for children and adults, and through programs and publications that improve the care of diabetes worldwide.

Postdoctoral Fellow

We have an immediate need for a Postdoctoral Fellow to further our research. Previous studies from the laboratory have demonstrated the critical importance of glucose 6-phosphate dehydrogenase and NADPH (the main cellular antioxidant) for normal cell growth and cell survival. Our research has also shown that certain pathophysiologic conditions such as diabetes are associated with decreased activity of G6PD and lower NADPH levels thus predisposing cells to cellular damage and death which leads to the development of diabetic complications. This Postdoctoral Fellow will work on extending our current research on G6PD, NADPH and diabetic complications.

Candidates must have an MD or Ph.D. and be interested in protein biochemistry, cell biology, and molecular biology. Both cell culture and animal models (including transgenic mouse models) are used. A particularly strong background in molecular biology and biochemistry is desirable.

Funding for this position is available through an NIH sponsored grant.

Send curriculum vitae and cover letter to: Dr. Robert C. Stanton, Chief, Renal Division, Joslin Diabetes Center, Boston, MA 02215. Fax: 617-732-2467, E-mail: robert.stanton@joslin.harvard.edu. For additional information, please visit: **www.joslin.org**. EOE.

Joslin Diabetes Center

www.joslin.org

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

Research Associateship Programs

Postdoctoral Research Awards
Senior Research Awards
Davies Teaching Fellowships
offered for research in residence at
US Government Laboratories

Opportunities for postdoctoral and senior research in all areas of science and engineering

- 350 awards for independent research at over 120 participating laboratories
- 12-month awards renewable for up to 3 years
- Annual stipend $36,000 to $65,000 - higher for senior researchers
- Relocation, professional travel, health insurance
- Annual application deadlines Feb. 1, May 1, Aug. 1, Nov. 1

Detailed program information, including instructions on how to apply, is available on the NRC Web site at :
www.national-academies.org/rap

Questions should be directed to :
National Research Council
TEL: (202) 334-2760
E-MAIL: rap@nas.edu

Qualified applicants will be reviewed without regard to race, religion, color, age, sex or national origin.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Sandia National Laboratories

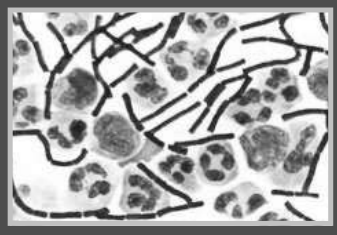
Postdoctoral Scientist
Molecular Immunology
Salary: $72,100

Sandia National Laboratories (SNL) located in Livermore, California, invites persons with training in Immunology and Molecular Biology to apply for a Postdoctoral position. Our current area of focus is the development of novel methods for the study of the innate immune response in macrophages. Candidates should possess a Ph.D. in Immunology or a related discipline and have experience in cell signaling. Successful candidates will be self-motivated and capable of working independently as well as with a multidisciplinary team of scientists and engineers at SNL and other institutions. The ability to obtain a DOE clearance is required.

For consideration you must complete an application online at www.sandia.gov. **Req #054673**. In addition, send curriculum vitae, names and contact information for three references, a description of research accomplishments and interests, along with up to three reprints to: **Dr. Todd W. Lane, Biosystems Research Department, Sandia National Labs, P.O. Box 969, MS 9951, Livermore, CA 945510969 or E-mail: twlane@sandia.gov**.

Equal Opportunity Employer. M/F/D/V.

BioDefense &
Infectious Diseases



Postdoctoral Fellowships
are available for U.S. citizens and permanent residents in the Interdisciplinary BioDefense and Infectious Diseases Training Programs.

Research Areas Include:
Anthrax cellular intoxication
Tularemia pathogenesis and vaccines
Ebola glycoprotein function
Poxvirus immunology
Enterics (Amebiasis, cryptosporidiosis, EHEC)
Respiratory viruses (Influenza, RSV & coronavirus)
Burkholderia pathogenesis
Innate & Adaptive immunity

UNIVERSITY of VIRGINIA HEALTH SYSTEM

For more information please see our web page at:
www.healthsystem.virginia.edu/inf-diseases/
or
contact us directly at:
medgpo13@virginia.edu

The University of Virginia is an equal opportunity/affirmative action employer. Members of underrepresented minority groups are encouraged to apply.

FNP
Fundacja na rzecz Nauki Polskiej

The Foundation for Polish Science

Call for Postdoctorates in HOMING Programme

The Foundation for Polish Science is pleased to announce a new programme for Ph.D.s at the early stage of their careers who intend to return to Poland and work in a Polish research entity.

Ph.D.s who are awardees of any similar return program or a program for international cooperation by a foreign organization are particularly encouraged to submit their application. The first **deadline is April 30, 2006**. Detailed information and forms accessible on **www.fnp.org.pl**

POSTDOCTORAL OPPORTUNITIES

NHLBI Training Center In Molecular Cardiology

Our NIH-funded postdoctoral training program in Molecular Cardiology, directed by Drs. Michael Schneider and Doug Mann, is available to outstanding MDs and PhDs alike. Areas of particular excellence include cell and gene therapy for cardiovascular disease, human genetics, myocardial ischemia and inflammation, atherosclerosis and lipoprotein research, cardiac development, and heart failure.

More than two dozen distinguished mentors are available. Our research is supported principally by the NIH, including large multi-investigator NHLBI Program Project Grants in cardiovascular gene therapy, genetics of congenital heart disease, myocardial ischemia, and cardiovascular development. Work on cell therapy for cardiac repair is supported by an NHLBI Specialized Center for Cell-Based Therapy for Heart, Lung, and Blood Diseases, and by a Transatlantic Network of Excellence for Cardiovascular Research from the Fondation Leducq.

Molecular Cardiology research is housed by Baylor College of Medicine's main campus, our adjacent clinical facilities (Texas Heart Institute, St. Luke's Episcopal Hospital, Texas Children's Hospital, The Baylor Clinic, Ben Taub General Hospital, Michael E. DeBakey Veterans Affairs Medical Center) and other contiguous participating institutions. The 170,000 sq. ft. Margaret M. Alkek Building for Biomedical Research, to be completed in late 2006, will house expanded space for cardiovascular research, diabetes, metabolism, and enabling technologies.

Physician-scientists seeking a combined fellowship in Clinical Cardiology (24 months) plus Molecular Cardiology research (24-36 months) should contact the Section of Cardiology directly: **Sonia Fuentes, Fellowship Coordinator, sfuentes@bcm.tmc.edu**. All other candidates for post-doctoral research in Molecular Cardiology (PhDs, or physicians taking a training sequence alternative to the above) should send their inquiries to **Michael D. Schneider, MD, Program Director, michaels@bcm.tmc.edu**.

Candidates must be citizens or non-citizen nationals of the US, or have been lawfully admitted for permanent residence. Baylor College of Medicine is an Equal Opportunity/ Affirmative Action/Equal Access Employer.

Leadership Opportunity in Bio-Engineering at Michigan Technological University

James and Lorna Mack Chair in Bio-Engineering

Michigan Tech's Department of Chemical Engineering has identified bio-engineering, bio-processing, and biotechnology as focus areas for growth. The Mack Chair holder will provide a strategic direction for interdisciplinary research in these emerging areas. The holder of the Chair will have the opportunity to collaborate with colleagues across Michigan Tech's campus, including our nationally renowned School of Forest Resources and Environmental Science, our Sustainable Futures Institute, and our Departments of Civil and Environmental Engineering and Biomedical Engineering. Responsibilities of the Mack Chair holder will include establishing a nationally recognized research program in bio-engineering at Michigan Tech, providing leadership for educational programs in this area, and teaching graduate and possibly undergraduate courses to support these research efforts. Opportunities for departmental governance also exist. Applicants with significant industrial experience are encouraged to apply.

Minimum requirements for the Mack Chair include an earned doctorate in Chemical Engineering, Bio-engineering, or any closely related field, and a professional stature meriting appointment as a tenured professor in the department. Application packages should include a complete CV, a vision statement for the position, and names of at least three references. Inquiries and applications should be directed to:

Ms. Christine Abramson
Dept. Coordinator
MTU Dept. of Chemical Engineering
203 Chem-Sci
1400 Townsend Drive
Houghton, MI 49931-1295

Michigan Tech is located in Michigan's scenic Upper Peninsula with abundant opportunities for outdoor recreation. The campus is one of the safest in the nation and the local community provides excellent resources conducive to quality family life. For more information regarding the university or the department, please visit **www.mtu.edu**.

Michigan Technological University is an Equal Opportunity Educational Institution/Equal Opportunity Employer.

POSTDOCTORAL OPPORTUNITIES

SPIRE

POSTDOCTORAL FELLOWSHIP PROGRAM

SPIRE (Seeding Postdoctoral Innovators in Research and Education) is an innovative postdoctoral fellowship program at The University of North Carolina at Chapel Hill.

SPIRE provides a multi-dimensional experience that enables Fellows to achieve research and teaching goals. This holistic approach allows Fellows to prepare for and succeed in the academic careers of their choosing and increases diversity in science professions.

SPIRE Fellows do everything traditional research postdocs do and more…

RESEARCH: Pursue independent research in laboratories covering a variety of disciplines.

TEACHING: Develop and teach courses at historically minority universities in North Carolina.

PROFESSIONAL DEVELOPMENT: Acquire workplace skills through science education workshops, student mentoring, technology-supported learning and other opportunities.

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THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

SPIRE is administered through UNC-Chapel Hill's Institute for Science Learning.

UNIVERSITY OF PENNSYLVANIA

The Basic Urology Research Laboratory in the Division of Urology at the University of Pennsylvania* is seeking highly motivated cell/molecular biologists, biochemists or physiologists for positions as postdoctoral fellows (Ph.D., D.V.M.-Ph.D., M.D.-Ph.D., or D.V.M.) and Research Associates. Our training program emphasizes translational research and a molecular biologic approach to understanding the pathogenetic mechanisms of urologic diseases. The fellows will have exposure and opportunities to translate basic science information into new diagnostic, preventive and therapeutic strategies. The rapid and effective translation of basic scientific discoveries is greatly facilitated by mentoring from basic and clinical scientists. Prospective candidates for the NIH-sponsored postdoctoral fellowship require U.S. citizenship or immigrant status, and should be recent graduates (< 3 years).

Our current research interests include (1) investigating the regulatory role of caldesmon in smooth muscle structure and function by disrupting gene expression using RNA interference (RNAi) and gene knock-out technologies, (2) alterations of intracellular kinases, phosphatases, and anchoring proteins in smooth muscle in urologic diseases, (3) smooth muscle signaling and contractile mechanisms and regulation in diabetes and smooth muscle remodeling, (4) bladder and prostate cancer, (5) gene therapy and (6) extracellular matrix. Interested candidates should forward their CV and the names of two references to: **Dr. Samuel K. Chacko, Director of Basic Urologic Research, 3010 Ravdin-Courtyard, HUP, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104. Fax: (215) 349-5026; E-mail: chackosk@mail.med.upenn.edu.** Start date is open.

**An Affirmative Action/ Equal Opportunity Employer.*

Associate Director for Basic Research
UNMC Eppley Cancer Center

The University of Nebraska Medical Center (UNMC) Eppley Cancer Center, a National Cancer Institute-designated Cancer Center, seeks outstanding candidates for the position of Associate Director for Basic Research. This Associate Director position will include a tenured appointment with academic rank commensurate with experience.

The successful applicant will be responsible for the overall direction and development of the Cancer Center's basic research programs. Responsibilities include maintaining an independent research program and fostering the continued development of basic research programs and interdisciplinary collaborations. This person will advise the Director on promising areas of research, provide direction to faculty members in pursuing research objectives, and be responsible for the Cancer Center's basic research shared facilities.

The UNMC Eppley Cancer Center is in a dynamic growth phase and committed to expansion of all its research programs. Growth in the cancer research programs is aided by generous support from the Nebraska Tobacco Settlement Biomedical Research Funds. With a strong commitment of both public and private funds, UNMC has made strategic investments in its research infrastructure with the addition of the Durham Research Center and the Lied Transplant Center which provide state of the art laboratory and clinical space for cancer research. UNMC plans to add another 242,000 square foot research building next year which will provide for continued growth of the Cancer Center.

Applicants should have a history of significant peer-reviewed funding, strong interpersonal and communication skills, and evidence of successful scientific collaborations. Experience in a leadership position within an NCI-designated Cancer Center is preferred. The position includes a generous start-up package and a primary appointment in the Eppley Institute for Research in Cancer and Allied Diseases.

Candidates should have a Ph.D. and/or M.D. degree. Applicants can apply online to position **# 1015** at **https://jobs.unmc.edu**. Additional information can be found at **http://www.unmc.edu/cancercenter/**. Candidates should forward a minimum of 3 letters of reference to:

Kenneth H. Cowan, M.D., Ph.D.
Director, Eppley Institute for Research in Cancer
Director, UNMC Eppley Cancer Center
University of Nebraska Medical Center
986805 Nebraska Medical Center
Omaha, NE 68198-6805
kcowan@unmc.edu

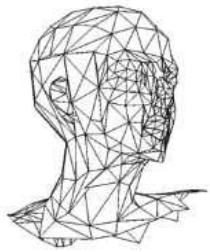
The University of Nebraska Medical Center is an Equal Opportunity Employer.

POSTDOC OPPORTUNITIES

Question
Explore
Research
Discover

University of Minnesota
Craniofacial Research Training
(MinnCResT) Program
NIDCR POST-DOCTORAL
FELLOWSHIPS
in Craniofacial, Dental and
Oral Health Research

Full support fellowships for post-doctoral interdisciplinary research training are available in one of 90 research laboratories. Applicants must have earned a PhD or DDS degree and be a US citizen or resident alien.



Apply online at:
www.mncrest.umn.edu

DIRECTOR
Center for Integrated Research in Cognitive and Neural Sciences
Southern Illinois University Carbondale

Southern Illinois University Carbondale (SIUC) invites applications for the Director of the newly established Center for Integrated Research in Cognitive and Neural Sciences (CIR-CNS). CIR-CNS is an interdisciplinary initiative that will build on existing researchers on the Carbondale and Springfield campuses as well as new recruits. The Director will have a unique opportunity to establish and promote multidisciplinary research groups and a training program in the integrated neural and cognitive/affective sciences. The Director of CIR-CNS will be offered a tenure-track appointment at the associate or full professor rank in the Department of Physiology with a 12-month state-funded salary, generous start-up funds, and spacious research facilities.

The candidate must have a doctoral degree, a strong publication record, and experience working with multidisciplinary research groups. The ideal candidate should have an internationally recognized and funded research program in an area of molecular, cellular, developmental, systems, or cognitive neuroscience and leadership qualities to promote collaboration and ensure vigorous growth of the Center in the full range of cognitive and neural sciences.

Applicants should submit a cover letter highlighting their interests and qualifications, curriculum vitae, research summary, and contact information for 5 persons qualified and willing to discuss the applicant's abilities to fill this position. Applications may be submitted electronically in PDF or RTF format to: **physiology@siumed.edu** or submit by regular mail to: **CIR-CNS Director Search Committee, Department of Physiology, School of Medicine, Southern Illinois University Carbondale, 1135 Lincoln Drive, Carbondale, IL 62901-6512.** Applications will be reviewed beginning **March 24, 2006** and continue until the position is filled. Information on the position, the departments, and participants affiliated with the Center can be found on the website: **www.siumed.edu/physiology/index.php?action=director**.

Women and minority applicants are encouraged to apply. SIUC is an Affirmative Action/Equal Opportunity Employer that strives to enhance its ability to develop a diverse faculty and staff to increase its potential to serve a diverse student population. This is a security-sensitive position. Before any offer of employment is made the university will conduct a pre-employment background investigation, which includes a criminal background check.

CHILDREN'S HOSPITAL BOSTON
HARVARD MEDICAL SCHOOL

Developmental Neuroscience
Assistant Professor

Applications are being considered for two full-time, tenure-track positions at Children's Hospital Boston and Harvard Medical School. The successful candidate will hold either a PhD or MD degree and will join an interactive research team in the Neurobiology Program directed by **Michael E. Greenberg, PhD,** and Department of Neurology, Children's Hospital. This program resides within a very strong and collegial research community in neuroscience and related disciplines throughout the Harvard Medical Area. Successful candidates will have research interests in developmental neuroscience with relevance to the function, development, or pathology of the nervous system, broadly defined. Modern laboratory space is available in newly renovated Enders Research Building. We seek outstanding scientists to establish a vigorous research program and form productive interactions with colleagues and other scientists at the institution. The investigators will hold both Children's Hospital Boston and Harvard Medical School faculty appointments.

Please submit a current CV, a 2- or 3-page description of research interests and directions, and three to five reference letters. Materials should be sent by March 15, 2006 to:

Neuroscience Search Committee
Attn: Xi He, Ph.D. c/o Diana Philips
Enders 260, Children's Hospital Boston
300 Longwood Avenue
Boston, MA 02115

Equal Opportunity/Affirmative Action Employer.

Director

MRC Laboratory of Molecular Biology

For over 50 years, the MRC Laboratory of Molecular Biology has produced world-class research and has been an outstanding source of discoveries in the broad field of fundamental biology. The primary goal of the Laboratory is to understand biological processes at the molecular level through application of a wide range of methods and techniques. In recent years, there have been major achievements in the fields of structural biology, cell and neurobiology, immunology and cancer biology, synthetic and computational biology, and genome sequencing. In addition, key areas of the research have led to very successful exploitation and to new therapies, most notably those based on monoclonal antibodies.

The continuing excellence of the research is underpinned by long term funding from the MRC, high quality scientific infrastructure and equipment, and a collaborative ethos. It also provides an excellent training environment for PhD students and postdoctoral fellows. Taken together, the Laboratory has a unique scientific culture, enabling ground-breaking discoveries and exciting innovation. Capital funding has been secured for a major new building on the Addenbrooke's campus, for which construction will commence in 2007.

The current Director, Richard Henderson FRS, is stepping down later this year, and we are now seeking his successor.

The successful candidate will have an outstanding scientific record in their field, with a reputation for making major breakthroughs in the life sciences. Funding will be provided to support their own research programme.

The new Director will be expected to work with the Heads of Divisions to lead the Laboratory in the next phase of its development, to set the overarching scientific strategy, and to determine priorities for the future, taking advantage of the opportunities presented by the new building. The role will require high quality management skills, with responsibilities including career development at all levels, resources, technology transfer and promotion of external relationships.

The appointment salary and other terms and conditions are negotiable. Relocation assistance will be provided.

The MRC is an Equal Opportunities Employer.

For further information and to discuss your interest in confidence, please contact Dr Kevin Young on +44 (0)1707 280819 or email 05536@theRSAgroup.com

Please quote the Ref: 05536N.

RSA Search & Selection
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The Melon Ground, Hatfield Park, Hatfield, Herts AL9 5NB
Tel 44 (0)1707 259333 Fax 44 (0)1707 271366 rsvp@theRSAgroup.com www.rsajobs.com

Health Research in a Changing World

Fighting Diseases and Improving Lives

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Are you ready for an exciting career that could help improve millions of lives around the world? Then consider joining the scientific and medical forces at NIAID. As part of the Division of Microbiology and Infectious Diseases (DMID) at NIAID, the Parasitology and International Programs Branch (PIPB) is responsible for planning and conducting programs of extramural research aimed at understanding the biology of protozoan and helminth parasites and their interaction with the human host as well as their vectors and intermediate hosts. PIPB/DMID has the following scientific opportunities available:

Program Officer/Medical Officer

As a Program/Medical Officer, the selected candidate will provide leadership and scientific/medical expertise and guidance in the planning, development, implementation and evaluation of basic and clinical research concepts, projects and initiatives to appropriate advisory groups; identify opportunities and problem areas, research gaps and relevant program needs and make recommendations for and facilitate new research efforts, clinical studies, clinical trials or other initiatives; and communicate with grantees/contractors, cooperative group members/representatives and others on policy interpretation, merit review and evaluation processes and procedures, and on decisions, concerns or other issues/matters of a medical/scientific nature. The selected candidate will also oversee and advise on preclinical development of candidate vaccines for parasitic diseases. In order to be considered for this position, applicants should have experience in basic and/or clinical research to examine the causes, diagnosis, treatment and prevention of infectious diseases; research on bacteriology, mycology, virology, or research on parasitic and other tropical diseases or vector biology is required. Experience in vaccine development and/or project management is highly desirable. For the Program Officer position, a Ph.D. and relevant experience are highly desirable. The selected candidate must posses an M.D. to be considered for the Medical Officer position.
To apply for the Program Officer vacancy, please visit
http://usajobs.opm.gov.
Vacancy number: NIAID-05-102836
GS-403/601-13/14 Salary: $74,782-$114,882
Open: 12/5/05-3/3/06

To apply for the Medical Officer vacancy, please visit
http://usajobs.opm.gov.
Vacancy number: NIAID-05-102836A
GS-602-13/14 Salary: $79,521-$114,882
Open: 12/5/05-3/3/06
In addition to the base salary, a Physician Comparability Allowance up to $30,000 per annum may be paid.

Applications must be submitted to Nolan Jones, Human Resource Specialist, 301-402-0957

Program Officer

As a Program Officer, the selected candidate will provide leadership and scientific expertise and guidance in the planning, development, implementation and evaluation of basic and clinical research concepts, projects and initiatives to appropriate advisory groups; identify opportunities and problem areas, research gaps and relevant program needs and make recommendations for and facilitate new research efforts; and communicate with grantees/contractors, cooperative group members/representatives and others on policy interpretation, merit review and evaluation processes and procedures, and on decisions, concerns or other issues/matters of a medical/scientific nature. The selected candidate will provide scientific and programmatic oversight and advice for research involving basic aspects of parasite biology, including genomics, functional genomics, molecular biology and biochemistry, and cell biology of protozoan and helminthic parasites. In order to be considered as Program Officer, applicants should have experience in basic and/or clinical research to examine the causes, diagnosis, treatment and prevention of infectious diseases; research on bacteriology, mycology, virology, or other viral diseases or research on parasitic and other tropical diseases and vector biology is required. Research experience at the Ph.D. and postdoctoral level is highly desirable, including but not limited to molecular biology and/or biochemistry.
To apply for the Program Officer vacancy, please visit
http://usajobs.opm.gov.
Vacancy number: NIAID-05-102837
GS-403/601-13/14 Salary: $74,782-$114,882
Open: 12/5/05-3/3/06
Applications must be submitted to Nolan Jones, Human Resource Specialist, 301-402-0957

To have your resume reviewed and for other opportunities, please visit: http://healthresearch.niaid.nih.gov/science and click "submit your resume"

DHHS and NIH are Proud to be Equal Opportunity Employers

WWW.NIH.GOV

NIMH
National Institute of Mental Health

STAFF SCIENTIST NEUROIMAGING RESEARCH POSITION NATIONAL INSTITUTE OF MENTAL HEALTH

The National Institute of Mental Health (NIMH), Division of Intramural Research Programs, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is recruiting a staff scientist to join the Section on Integrated Neuroimaging, in the Clinical Brain Disorders Branch. This organization is housed at one of the premier research sites in the U.S., the 300 acre Bethesda campus of the NIH, near Washington D.C. with state-of-the-art neuroimaging facilities (MRI, PET and MEG) dedicated to research. Minimum qualifications are a Ph.D. degree, post-doctoral training, strong publication record, and demonstrated expertise in analysis (computational and statistical methods) and synthesis of neuroimaging data. The successful candidate will be part of a multidisciplinary team using neuroimaging to map brain activity as well as genetic and neurochemical mechanisms associated with normal higher cognitive function as well as dysfunction in neuropsychiatric illnesses such as schizophrenia, those with genetic sources of cognitive dysfunction such as Williams syndrome, and other conditions such as normal aging. In addition to collaborative work within the team, there is opportunity for outstanding candidates to develop their own projects within the Section. Possible areas of concentration include 1) neurofunctional substrate of higher cognitive function, particularly as regards working memory and frontal lobe, 2) neurofunctional bases of neuropsychiatric illnesses, 3) computational neuroscience (statistical and systems approaches), and 4) neurochemical underpinnings of higher cognitive function and dysfunction. Stipends are competitive and depend on level of experience. Send letter of interest outlining experience and research goals, CV, and three letters of recommendation ASAP to: Karen F. Berman, M.D.; NIH Building 10, Rm 4C101; 9000 Rockville Pike; Bethesda MD 20892-1365 USA. Phone: (301) 496-7603; FAX: (301) 496-7437. E-mail: karen.berman@nih.gov.

Post-doctoral Fellow or Research Fellow
National Institute of Allergy and Infectious Diseases

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for a Post-doctoral Fellow or Research Fellow. The position is available in the Bacterial Toxins and Therapeutics Section of the newly formed Laboratory of Bacterial Diseases (LBD). The LBD will be located in new and well-equipped facilities on the main NIH campus. Scientists with a M.D., Ph.D., or DVM are eligible. The Research activity involves (1) characterization of bacterial virulence factors, particularly toxins, proteases, and hemolysins, in both cultured cells and animal infection models; (2) structure-function analyses of protein toxins; (3) study of gene regulation in Bacillus anthracis; (4) identification of new candidate vaccines and therapeutics for anthrax; and (5) development of toxin variants that target specific cell types, e.g., malignant cells. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to development of vaccines and therapeutics, and it provides excellent training for newly graduated Ph.D. scientists, for postdoctoral scientists, and for MD's at all levels of training who plan a career in research in infectious diseases. The salary range for Post-doctoral Fellows is $38,500-56,900, depending on experience. Research Fellow applicants should have three or more years of post-doctoral experience; the salary range is $40,974-72,990. Applicants with an MD degree are eligible for the NIH Loan Repayment Program. Applicants should send their curriculum vitae, a letter of interest, and names and addresses of three (3) references to **Stephen Leppla, 30 Convent Drive, MSC 4349, Building 30, Room 303, Bethesda, MD 20892-4349, FAX: (301) 480-0326, email: sleppla@niaid.nih.gov**

NATIONAL CANCER INSTITUTE

Gene Regulation and Transcription Control In *E. Coli and H. Pylori*

A postdoctoral position is available in a research program focused on global gene regulation and transcription control using simple model bacterial systems including *E. coli* and *H. pylori*. Of particular interest are the transcription apparatus and/or architecture that responds to environmental cues, and transcription control in pathogenesis. We use an integrated approach of genetics, biochemistry, molecular and cell biology to investigate the issues.

Applicants must have a Ph.D. and/or an M.D. with less than five years of postdoctoral experience, and have a strong background in molecular biology, biochemistry, and/or microbiology. The salary range is commensurate with experience.

To apply, send e-mail with CV, bibliography, the names of three references with phone numbers and e-mail addresses, and a cover letter briefly describing research experience, interests, and career goals to:

Ding Jun Jin, Ph.D, Transcription Control Section, Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute-Frederick, NIH, Phone: 301 846-7684, Email: djjin@helix.nih.gov, http://ccr.cancer.gov/staff/staff.asp?profileid=5787

Postdoctoral, Research and Clinical Fellowships at the National Institutes of Health

www.training.nih.gov/pdopenings

www.training.nih.gov/clinopenings

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HEALTH EFFECTS INSTITUTE

Science Positions

HEI seeks strong candidates at various levels to strengthen its scientific staff. HEI is a nonprofit organization designed to provide impartial, relevant scientific information about the health effects of air pollution to decisionmakers in government and industry. Its core funds come equally from the U.S. Environmental Protection Agency and industry; HEI also receives funds for some projects from other governments, industries, and foundations. HEI is funding a broad range of epidemiologic, toxicologic, and other research at universities and research centers in many countries. Priorities in HEI's strategic plan are: (1) health effects of air pollution; (2) assessing emerging technologies; (3) evaluating the public health impact of actions to improve air quality; (4) and international health effects research. Additional information about HEI's work can be found at **www.healtheffects.org**.

Epidemiologists/Biostatisticians: HEI has 2 openings for epidemiologists or biostatisticians to work with HEI 's Research and Review Committees and Scientific Staff to develop and implement HEI's research program on air pollution and health. We are looking for scientists with a strong foundation in epidemiologic and statistical methods who are interested in the health effects of air pollution. Experience in air pollution epidemiology is desirable, but not required. These scientists would plan future research directions, participate in selecting and overseeing studies, review and write commentaries on final reports from HEI investigators, and contribute to literature reviews on critical issues. Interesting responsibilities on the horizon are:

- Initiating and overseeing a major research program to investigate which components or sources of particulate air pollution pose greater health risks than others.
- Managing ongoing "accountability" studies in several countries that are measuring the exposure and health impacts of air quality actions designed to improve public health.
- Reviewing final reports of HEI-funded epidemiologic studies on health effects of air pollution from traffic and from particulate air pollution in North America and Europe, and on accountability studies, and writing commentaries, to be published with the reports, that describe strengths, limitations, scientific contributions, and relevance to human health effects of the research in terms understandable to a diverse audience.
- There are also periodically opportunities to contribute as an author of reviews of critical scientific issues.

We seek to hire at the Staff Scientist and Senior Scientist levels. Both require a doctoral degree in epidemiology or biostatistics; the Senior Scientist position requires 5+ years of subsequent experience in epidemiologic research. Experience in project management is a priority. Good written and oral communication skills are essential. Experience on how research is used in public health and/or policy settings is desirable.

Research Assistants: HEI seeks to hire 1 or 2 Research Assistants who would assist Staff Scientists by gathering and organizing information and drafting summaries on topics relevant to current projects. Backgrounds in biology, chemistry, toxicology, epidemiology, mechanical engineering, environmental science, and similar disciplines are of interest. These positions offer an opportunity for someone with initial science training to obtain experience as she or he considers next steps in education and career development.

As part of one of these positions, in addition to work described above, we are also looking for someone to assist in managing HEI's quality assurance (QA) program on funded research, which is conducted by external QA auditors. Previous QA experience is desirable, but not required; HEI is willing to pay for QA training. Applicants interested in the QA aspect should have some research experience. The Research Assistant position requires a bachelor's or master's degree in one of the disciplines listed above.

Applying for These Positions: For all positions, interested applicants should submit a letter, resume, and writing samples no later than **April 30, 2006**. Please send application material to: **Ms. Terésa Fasulo, Manager of Science Administration, Health Effects Institute, Charlestown Navy Yard, 120 Second Avenue, Boston MA 02129, tfasulo@healtheffects.org**.

HEI is an Equal Opportunity Employer.

U.S. Environmental Protection Agency
Office of Research and Development
National Center for
Environmental Assessment (NCEA)

Supv. Biologist/Toxicologist/Health Scientist/Physical Scientist/Mathematical Statistician

Ez hire Announcement #RTP-DE-2006-0048 or RTP-MP-2006-0080

The U.S. Environmental Protection Agency is seeking highly qualified applicants for two Branch Chief positions with the National Center for Environmental Assessment (**http://cfpub.epa.gov/ncea/**) which are located in Cincinnati, Ohio. Duties include supervision and leadership of an interdisciplinary team of scientists conducting high-profile human health and ecological assessments and developing cutting-edge risk assessment methods, with emphasis on water quality and hazardous waste.

Excellent benefits: The selected candidate will be eligible for a full benefits package, including paid relocation, health insurance, life insurance, retirement, and vacation and sick leave. This is a permanent, full time position. U.S. citizenship is required.

Salary Range: The salary range is $91,080 to $139,275 (GS 14/15) per year, commensurate with qualifications.

Qualifications: A bachelor's degree (or higher) is required. Desirable applicants will have an advanced degree and demonstrated experience in conducting research and leading research teams in environmental health, toxicology, biology, physical science, mathematical statistics, or a related field.

How to Apply: Applicants should apply through Ezhire at **http://www.epa.gov/ezhire** Select apply for jobs. If you are already registered in Ezhire@EPA system, access the vacancy announcement through Registered Users. Otherwise, select New Users and complete the registration process. The vacancy announcement will be open through March 13, 2006. Application materials must be submitted with 48 hours from the closing date of the announcement. You need to submit the additional documentation described in the full text vacancy. Questions regarding this vacancy may be directed to **Joann Kelleher, Human Resources Management Division** at **kelleher.joann@epa.gov**.

The US EPA is an Equal Opportunity Employer.

Profectus Biosciences, Inc. ("Profectus") is a start-up biotechnology company located in the suburbs of Baltimore, Maryland. Our focus is the development of preventative and therapeutic technologies intended to reduce the morbidity and mortality caused by viral diseases, in particular, Human Immunodeficiency Virus (HIV). Profectus is looking for individuals who would be interested in developing vaccines and antibody based antiviral therapeutics. Candidates must be capable of working successfully either independently or as a member of a team and exhibit superb written and verbal communication skills. Familiarity with GLP/GMP regulations are a plus. The candidate must also be lawfully permitted to work in the United States.

Profectus offers competitive compensation packages including medical/dental insurance, stock options, and 401K program based on experience. Profectus is an equal opportunity employer, dedicated to a policy of nondiscrimination in employment on the basis of race, religion, color, sex, national origin, age, marital status, sexual orientation, genetic information, citizenship, veterans' status, physical or mental disability that does not prohibit performance of essential job functions or any other basis protected by federal, or applicable state or local law.

Please send letter describing relevant experience and research interest along with current curriculum vitae to: **Dr Timothy Fouts, Profectus BioSciences, Inc., Techcenter at UMBC, 1450 South Rolling Road, Baltimore, MD 21227** or via email to **fouts@profectusbiosciences.com**.

Scientist: A Ph.D. or equivalent in molecular and cell biology, immunology, biochemistry with 3-5 years research laboratory experience in the study of T cell function, signaling and cell activation.

Scientist: A Ph.D. or equivalent in molecular and cell biology, immunology, biochemistry with 3-5 years research laboratory experience in the study of antibody responses to antigens and/or B-cell immunology.

Research Assistant (2 positions): MS or BS in molecular and cell biology, immunology, biochemistry with research laboratory experience handling primary blood cells, and live viruses are required. Candidates with advanced technical skills in FACS, immunochemistry, protein purification, molecular biology, tissue culture are desired.

I want more opportunities.

Senior Scientist - Cell Biology

Lund, Sweden

A Senior Scientist position is available with focus on Respiratory Epithelial Cell Biology. The successful candidate will have a strong Cell Biology background preferably in Respiratory Science. We look for an independent, experienced Scientist (PhD level) with Post-Doctoral training. Experience in primary cell cultures is highly meriting as is previous experience of the Pharmaceutical industry. The work will focus on finding new therapies for COPD and Asthma and will entail building knowledge around pathophysiology, development of epithelial models and evaluation of promising drug candidates. Teamwork is crucial and we require good communication skills, flexibility and scientific drive.

For further information please contact
Elisabet Wieslander, TeamLeader Cell Biology,
phone: +46 46 33 66 00.

Please send your application and CV marked "Ref. No. 09/06" no later than 6th of March, 2006 via. www.astrazeneca.se. We will only handle applications received via our website.

Inhalation in vivo pharmacologist

Lund, Sweden

A challenging and varied job in the in vivo Section in Lund is waiting for a competent, independent, dynamic and lab based research scientist who will be a key driver of inhalation technology and lung function measurement. We offer an exciting work environment with great possibilities for development in an efficient section. You possess a candidate degree in biology, pharmacology, medicine or equivalent. Preferably with expertise in the respiratory inflammation area.

For further information please contact
AZ switchboard, phone +46 46 33 60 00.

Please send your application and CV marked "Ref. No. 14/06" no later than 6th of March, 2006 via www.astrazeneca.se. We will only handle applications received via our website.

Further information regarding this and other available jobs can be found on our website.

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CHARITÉ

MDC MAX-DELBRUECK-CENTER FOR MOLECULAR MEDICINE (MDC) BERLIN-BUCH

The MAX DELBRUECK CENTER FOR MOLECULAR MEDICINE (MDC) BERLIN-BUCH and the Medical Faculty of the CHARITÉ - UNIVERSITÄTSMEDIZIN BERLIN (CHARITÉ) invite applications for the following position:

Full Professorship of Cardiovascular and Metabolic Diseases

(W3 BBesG)

Code number: Prof. 273/2006

The MDC Berlin-Buch is a biomedical research institute dedicated to interdisciplinary research in the areas of (i) Cardiovascular and Metabolic diseases, (ii) Cancer, and (iii) Function and Dysfunction of Nervous System.

The MDC is committed to expanding its impact in the field of **Cardiovascular and Metabolic Diseases** and is seeking applications from outstanding individuals with international reputation in relevant areas of research including **genetics, genomics or pathophysiology of organ (dys)function, vascular biology** or **metabolic diseases**.

Successful candidates will conduct visionary independent research, obtain extramural funding and engage in collaborative projects with groups at the MDC, the Gene Mapping Center and the Franz Volhard Clinic for Cardiovascular Diseases of the CHARITÉ.

The successful applicant will be scientific member of the MDC Berlin-Buch and of the Medical Faculty of the CHARITÉ. The position is affiliated with the MDC Berlin-Buch.

For further information about the CHARITÉ and the MDC Berlin-Buch please visit our web sites **http://www.charite.de or http://www.mdc-berlin.de**. For enquiries about the position please contact Thomas Willnow (**willnow@mdc-berlin.de**).

Applications should be sent within six weeks after publication of this advertisement including a curriculum vitae, list of publications, an outline of present and planned research and other relevant material (see: **http://www.charite.de/fakultaet/aktuelles/hinweise_professuren.html**)

either to

Prof. Dr. Martin Paul
Dean
Charité - Universitätsmedizin Berlin
10098 Berlin
Germany

or to

Prof. Dr. Walter Birchmeier
Scientific Director
Max Delbrueck Center for Molecular Medicine (MDC) Berlin-Buch
Robert-Rössle-Str. 10, 13125 Berlin-Buch
Germany

The CHARITÉ and the MDC Berlin-Buch are equal opportunity employers. The MDC Berlin-Buch is a member of the Helmholtz Association of National Research Centers, supported by the Federal Government of Germany and the Land Berlin.

UNIVERSITY of VIRGINIA HEALTH SYSTEM

ASSOCIATE OR FULL PROFESSOR

Bacterial Physiology, Genetics and/or Pathogenesis

The Department of Microbiology at the University of Virginia School of Medicine invites applications for a tenured or tenure-track faculty position at the rank of Associate or full Professor. Candidates should have a Ph.D. or M.D. degree and have a record of outstanding achievement in research and graduate and postgraduate training. The successful candidate will be expected to maintain an energetic and well-funded basic research program in aspects of bacterial physiology, genetics and/or pathogenesis. In addition he/she is expected to actively participate in Department and Medical School teaching. The successful candidate is expected to take a leadership role in the continuing development of outstanding basic and clinical research on microorganisms and human disease at the University of Virginia.

The Department of Microbiology offers state of the art research space, numerous core facilities for the support of molecular and cellular research, and a collegial and interactive faculty. Outstanding opportunities exist for collaborative research in both basic and clinical sciences, including programs in microbial pathogenesis, cellular microbiology and infectious diseases.

Interested applicants should provide a curriculum vita, brief statement of research interests, and arrange to have at least three letters of reference sent to:

Search Committee, Attention: Lynn McCutcheon
Department of Microbiology
University of Virginia Health System, Box 800734
Charlottesville, VA 22908

Fax: (434) 982-1071 - Email: lam8t@virginia.edu

Web: **http://www.healthsystem.virginia.edu/internet/microbiology/**

Review of applications will commence March 1, 2006.
The position will remain open until filled.

The University of Virginia is an Equal Opportunity/Affirmative Action Employer

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Research Biologists

Ocular Biology

La Jolla/San Diego, CA

Join our La Jolla Laboratories, Pfizer's center of research and development for diseases of the eye. As a member of our world-class Ocular Biology team, you'll have an opportunity to play a vital role in the successful discovery of new medicines for the treatment of Glaucoma, Diabetic Retinopathy and AMD. We have several openings at the BS/MS and PhD levels for talented scientists with in vivo ocular biology experience.

We offer competitive compensation, full benefits and talented professional colleagues… some of the best and brightest in the research field today. For a full description and to apply on-line, visit: **www.pfizer.com/careers** and search by one of the following Req numbers: **048529; 048530; 048532; 048812; 048548; 048813.**

Pfizer is proud to be an Equal Opportunity Employer and welcomes applications from people with different experiences, backgrounds and ethnicities.

PGRD
Pfizer Global Research & Development

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

BROWN

Center for Environmental Studies, Tenure Track Assistant Professor Sharpe Endowed Chair in Environmental Studies

The Center for Environmental Studies (CES) at Brown University seeks a faculty member for an endowed chair at the ASSISTANT PROFESSOR level with broad interests in environmental sciences as well as public policies related to environmental issues. This appointment will be tenure track and entail a joint appointment between CES and one of the following academic units: Community Health, Ecology and Evolutionary Biology, the Division of Engineering, Geological Sciences, Political Science, or Sociology, depending on the background and research record of the candidate. We seek candidates who can integrate basic and applied environmental sciences and work at different scales from local to regional and global.

Requirements include a PhD in an environmentally related discipline, a strong record of research and outreach, commitment to excellence in graduate and undergraduate teaching, and potential for interdisciplinary collaboration. The mission of the Center for Environmental Studies is to carry out interdisciplinary education, research, and outreach on a variety of topics related to the environment. CES interests encompass the natural sciences, social sciences, and public health. For more information about the CES visit **http://envstudies.brown.edu/env/index.php**.

To apply, please send a letter describing research, teaching, and outreach interests and the fit of the candidate with the CES, a current CV, and 3 letters of reference to: **Professor Osvaldo Sala, Director, Center for Environmental Studies, Box 1943, 135 Angell Street, Brown University, Providence, RI 02912**. For further inquiries, please contact **Osvaldo_Sala@Brown.edu**. Applications will be reviewed starting on **February 28, 2006** and accepted until the position is filled.

Brown University is an EEO/AA Employer.

New York University

SENIOR FACULTY POSITION IN CHEMISTRY

Department of Chemistry and Molecular Design Institute

FACULTY OF ARTS AND SCIENCE

The Department of Chemistry and the newly established Molecular Design Institute (MDI) at New York University invites applications for a tenured associate or full professor faculty appointment in supramolecular materials chemistry, preferably with expertise in synthetic organic or polymer chemistry. The anticipated start date is September 1, 2006, pending budgetary and administrative approval. The appointee will play an active role in the development of the MDI, an initiative within the Department of Chemistry that is part of the continuing expansion of faculty and facilities in the Faculty of Arts and Sciences at New York University.

Candidates should have an established record of excellence in research and teaching. All correspondence should be sent to: **Professor Michael D. Ward, Chair, Faculty Search Committee, Department of Chemistry, Faculty of Arts and Science, New York University, 100 Washington Square East, New York, NY 10003.** Applicants are welcome to visit *http://www.nyu.edu/pages/chemistry* for more details.

NYU is an Equal Opportunity/Affirmative Action Employer.

Assistant/Associate Professor in Mammary Gland Biology University of California, Davis

The Department of Animal Science in the College of Agricultural and Environmental Sciences seeks applicants for an Assistant/Associate Professor in Mammary Gland Biology with teaching, research and outreach responsibilities consistent with the mission of the California Agricultural Experiment Station. We seek outstanding applicants with a Ph.D. or equivalent degree. Post-doctoral experience is preferred for candidates at the Assistant level. These educational experiences should have emphasized mammary gland biology and prepared the candidate for the study of mammary gland biology using cutting-edge biotechnological approaches such as functional genomics, proteomics, or metabolomics. Appointees are expected to develop an extramurally funded research program emphasizing mammary gland function. The appointee is required to teach an undergraduate course in the biology of lactation with additional contributions to departmental courses and graduate education are expected. Mentoring of graduate students, undergraduate student advising, participation in outreach programs, curricular development, and performance of University service are also expected. The successful candidate is expected to develop a research and teaching program relevant to the California dairy industry, the state's largest industry, and contribute to outreach consistent with the missions of the Agricultural Experiment Station. The position is a nine-month tenure track appointment; eleven-month term employment to be offered and continued based upon academic personnel review.

The position will be available on or about September 1, 2006. Applicants should submit a CV including list of publications, transcripts (for Ph.D. awards within 5 yr), a detailed description of research and teaching accomplishments, and statement of future plans, copies of relevant in-press publications and manuscripts, and the names and contact information of three to five references to: **Professor A.M. Oberbauer, Search Committee Chair, Department of Animal Science, One Shields Avenue, University of California, Davis, CA 95616, telephone (530) 752-4997, amoberbauer@ucdavis.edu**. Open until filled but to ensure consideration, applications should be received by **March 31, 2006**. A more detailed job description is available at **http://animalscience.ucdavis.edu/**. E-mail applications will not be considered.

University of Hawai'i MĀNOA

Director, Pacific Biosciences Research Center

The University of Hawai'i at Mānoa invites nominations and applications for a dynamic and visionary leader to serve as Director of the Pacific Biosciences Research Center (PBRC). PBRC is a college-level, organized-research unit which serves as administrative home to laboratories and centers of research in interdisciplinary research and training in the biological sciences. Research emphases currently include cellular, developmental and molecular biology; marine biology; Hawaiian evolutionary and conservation biology; neuro-behavioral biology; and molecular endocrinology. In collaboration with academic units at the University of Hawai'i at Mānoa, PBRC also supports training in biosciences for undergraduates, graduate students, postdoctoral fellows and junior faculty.

The University of Hawai'i at Mānoa campus is the only doctoral/research-extensive university in Hawai'i. By virtue of its culture and geographic location, the University of Hawai'i at Mānoa plays an important role in providing Asian, Pacific, and Hawaiian perspectives to the higher education experience.

Nominations and applications are being accepted for the position. Review of candidates is ongoing; to insure full consideration, applications should be received by **March 27, 2006**. Recruitment will continue until the position is filled. Each candidate must submit a cover letter summarizing his/her interests and qualifications for the position, a current resume, and the names of three (3) professional references including postal and e-mail addresses and telephone numbers. For more information about the University of Hawai'i at Mānoa, please go to www.uhm.hawaii.edu. For a job description and specific application/nomination requirements, please go to **www.hawaii.edu/executivesearch/pbrc** or **http://workatuh.hawaii.edu**. Applications and nominations should be submitted to:

Office of the Vice Chancellor
for Research and Graduate Education
Re: Director, Pacific Biosciences Research Center
2500 Campus Road, Hawaii Hall 211
Honolulu, HI 96822

Phone: (808)956-7837
Fax: (808)956-2751
E-mail: gko@hawaii.edu

The University of Hawai'i is an Equal Opportunity/Affirmative Action Institution and encourages applications from and nominations of women and minority candidates.

Positions in Natural and Applied Sciences

Bentley College announces an important initiative in Natural and Applied Sciences with the launching of strategic programs for science education and research in the context of business and business processes. Bentley is a national leader in business education, integrating the business curriculum with Arts and Sciences courses and a Liberal Studies Major that provides the broad perspectives and skills necessary for a lifetime of personal engagement and professional success. Three new programs in the Natural and Applied Sciences will expand education and research in the domains of Health Science and Industry, Consumer Product Dynamics, and Earth, Environment and Sustainable Development.

The Department is seeking applications for three tenure track positions beginning in 2006-2007. Successful applicants must be outstanding teachers who can engage undergraduate students with business interests and also perform innovative research in their fields of expertise. Collaborative research that leverages the school's business perspectives as well as existing expertise in information systems, data mining, and modeling is encouraged. Research interests that bridge science and industry are welcomed.

Assistant/Associate Professor in Physics or related disciplines: The successful applicant will contribute to the development of new curriculum in physics as well as its applications in the design, manufacture, function, or marketing of consumer and industrial products. A Ph.D. is required. Post-doctoral training, or prior professional experience, in the fields of consumer electronics, material science, systems analysis, or industrial research and development are preferred.

Assistant Professor in Epidemiology, Public Health, or Healthcare: The successful applicant will contribute to the development of new curriculum in human biology as well as applications in health and wellness, healthcare, and the biopharmaceutical industry. A PhD is required. Post-doctoral training, or prior professional experience, in areas of epidemiology, public health, healthcare, or healthcare regulation is preferred.

Associate/Assistant Professor in Psychology: The successful applicant will contribute to development of a revised curriculum that will provide students with a broad introduction to psychology as well as applications in development, health, social and industrial/organizational psychology, and cross-cultural understanding. A Ph.D. and experience with undergraduate psychology education is required. Professional interests in applied developmental psychology with an emphasis on human cognition and applications in health, industrial/organizational, or cross-cultural psychology is preferred.

Please visit **http://www.bentley.edu** for more information about Bentley. Applicants must submit a cover letter with the names and contact information for three references, a complete C.V. and a 2-page statement on teaching philosophy and research interests. Applications should be directed to: **Chair, Search Committee, Natural Sciences Department, Bentley, Waltham, MA 02452-4705**, or emailed to **NSSEARCH@bentley.edu**. Consideration of applications will begin immediately and continue until the positions are filled.

Bentley is an Equal Opportunity Employer, building strength through diversity.

Research Opportunities!

Affymetrix currently has several research and development positions available in our Santa Clara, CA Headquarters. Affymetrix, the source for molecular technologies to understand and improve life, invented the world's first microarray in 1989 and began selling the first commercial microarray in 1994.

OPPORTUNITIES EXIST FOR:

Forensic Scientist

You will work on novel forensics applications of high-density microarray technology, and develop assays based on Affymetrix genotyping and resequencing technologies that will enable use for forensics applications. Requires a Ph.D. in Molecular Biology, Genetics, Biochemistry, or related field and 1-3 years of post-graduate work.

Bioinformatics Scientist (2)

Requires a Ph.D. in Statistics, Computer Science, Bioinformatics, or related field, and computer and programming skills in JAVA, Perl, C++, Linux/Unix, and Windows.

Staff Scientist

Requires a Ph.D. in Molecular Biology, Genetics, Biochemistry, Physical Chemistry, or related field and 2+ years of relevant experience in DNA analysis, mammalian systems and standard molecular biology procedures and concepts.

Biostatistician

You will be responsible for the statistical analysis/synthesis of human transcriptome expression data, transcription factor binding site ChIP data and regions of chromatin modification ChIP data. Requires a Ph.D. in a computational field, including Bioinformatics, Statistics, Computer Science, Physics, Mathematics, Engineering, or Chemistry and a strong background in data analysis and the application of statistical methods.

To view complete job descriptions and to apply online, please visit:

http://www.affymetrix.com/corporate/careers

You may also e-mail your resume to: **connie_vanlieu@affymetrix.com**.

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POSTDOCTORAL FELLOW
Department of Biochemistry/Molecular Biology
Mayo Clinic College of Medicine
Mayo Clinic Arizona

A Postdoctoral Position is available to study the role of MUC1 in cancer. The successful candidate will analyze the molecular mechanisms of how MUC1 influences cellular growth, transformation, adhesion, metastasis and immune surveillance and employ transgenic and knock-out mouse models to study MUC1 in vivo function. The ideal candidate will have experience in cell and molecular biology and mouse models. He/she should be highly motivated with publications and excellent oral and written communication skills. Maximum consideration will be given to candidates with first author publications.

Please send curriculum vitae and bibliography, summary of past accomplishments, and the names of three references to:

Sandra J. Gendler, Ph.D.
Mayo Clinic College of Medicine
Mayo Clinic Arizona
13400 E. Shea Boulevard
Scottsdale, AZ 85259
E-mail: jrbjobs@mayo.edu

Mayo Clinic College of Medicine is a not-for-profit organization that integrates research with clinical practices and education in multi-campus environment. Mayo offers an attractive benefit package. Salary will be determined by the successful candidate's experience.

Mayo Clinic College of Medicine is an Affirmative Action and Equal Opportunity Employer and Educator.

POSTDOCTORAL POSITIONS
Georgetown Unversity Medical Center

Postdoctoral Position is available immediately to study signal transduction pathways associated with human cancer progression. The projects include molecular and biochemical analyses, isolation or novel genes, discovering DNA array gene expression profiling with bioinformatics, and clinical translation studies for gene therapy. Recent Ph.D. training in molecular or cellular biochemistry. Prefer qualified candidates who are motivated, creative, independent and experienced in these areas, and less than five years of postgraduate. Send curriculum vitae and names of three references to: **Mira Jung, Ph.D., Professor, Department of Radiation Medicine, Division of Radiation Research, Georgetown University Medical Center, 3970 Reservoir Road, NW, Room E211A, The Research Building, Washington, DC 20057-1482. E-mail: jungm@georgetown.edu; fax: 202-687-0400.**

Georgetown University is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL RESEARCH ASSOCIATE. Position available to design fluorescence sensors and caged inhibitors for MAP kinase signal transduction studies. Candidates should have experience in peptide chemistry, protein chemistry and molecular biology. Please respond to: **Kevin Dalby, College of Pharmacy, Division of Medicinal Chemistry, The University of Texas at Austin, 1 University Station A1935, 2409 University Avenue, Austin, TX 78712.** Applications will be accepted until suitable candidates are found. *The University of Texas at Austin is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION, HUMAN NEUROIMAGING, Miller Laboratory, Center for Mind and Brain, University of California at Davis. NIH-funded project investigating the neural bases of speech perception. Experience in fMRI and/or high-density EEG an advantage but not required. Position offers competitive salary and full health benefits. For more information, see **website: http://mindbrain.ucdavis.edu/content/Labs/Miller/Positions.**

POSITIONS OPEN

TWO ONE-YEAR LEAVE
REPLACEMENT POSITIONS
Prokaryotic Microbiology, Eukaryotic Genetics
Grinnell College
Department of Biology

Grinnell College invites applications for two one-year leave replacement positions at the rank of **ASSISTANT PROFESSOR** in the Department of Biology in the areas of prokaryotic microbiology and eukaryotic genetics. Positions begin in fall 2006, with the possibility of a second year extension for the microbiology position. A Ph.D. is required and a postdoctoral preferred. Successful candidates should be prepared to teach at all levels of an innovative undergraduate biology curriculum based on research-centered learning including molecules, cells, and organisms (biology 251) and at least one upper-level course in the candidate's specialty area. For more information about the Department see **website: http://www.grinnell.edu/academic/biology/.** In letters of application, candidates should discuss their interest in developing as a teacher and scholar in an undergraduate, liberal-arts environment that emphasizes close student-faculty interaction and values diversity. To be assured of full consideration, all application materials should be received by March 1, 2006. Electronic applications will not be accepted. Send your application, curriculum vitae, copies of undergraduate and graduate transcripts, and three letters of reference to: **Charles H. Sullivan, (specify Genetics or Microbiology Search Committee) Department of Biology, 1116 Eighth Avenue, Grinnell College, Grinnell, IA 50112-1690. E-mail: sullivac@grinnell.edu, telephone: 641-269-3042; fax: 641-269-4285.** For further information about Grinnell College, see our **website: http://www.grinnell.edu.**

Grinnell College is an Equal Opportunity/Affirmative Action Employer committed to attracting and retaining highly qualified individuals who collectively reflect the diversity of the nation. No applicant shall be discriminated against on the basis of race, national or ethnic origin, age, gender, sexual orientation, marital status, religion, creed, or disability.

THE JOHN CALDWELL MEEKER
POSTDOCTORAL FELLOW

Applications are invited for the position of the John Caldwell Meeker Postdoctoral Researcher in the Department of Geology at The Field Museum. The successful candidate will be expected to complement, and/or participate in, any one of the ongoing research programs in the Department of Geology. Research projects are being pursued in the areas of Vertebrate Paleontology, Invertebrate Paleontology, Paleobotany, and Meteoritics. Individual Curators and their research programs are featured on the Field Museum **website: (http://www.fieldmuseum.org/research_collections/geology/research.htm).**

A Ph.D. in any field of research represented in the Department of Geology is required. The term for this position is for a maximum of two years. The appointment is anticipated to begin September 1, 2006.

Please send a statement of research interests and experience, curriculum vitae including publications list, and names of three references references (with telephone numbers and e-mail addresses) to: **Olivier Rieppel, Chair, Department of Geology, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605-2496 U.S.A. E-mail: orieppel@fieldmuseum.org; telephone: 312-665-7630; fax: 312-665-7641.** Applications must be received by March 31, 2006; applications received after this date may be considered if the position has not been filled.

The Field Museum is an Equal Opportunity Employer.

ADVANCED POSTDOCTORAL ASSOCIATE

Experimental Pathologist to study colon and breast cancer, circadian clock and menstrual cycle coordination of growth and spread. Highly collaborative well funded laboratory with basic, translational, and clinical research opportunities. Salary commensurate with skills, experience.

(See **website: http:// www.scmedicalchronobiology.org.**) Send curriculum vitae and three letters of support to **e-mail: william.hrushesky@va.gov.**

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION
in Oral Biology

The University at Buffalo Department of Oral Biology invites applications for a full-time tenure-track faculty position at the Professor/Associate Professor/Assistant Professor level. We are seeking outstanding individuals capable of establishing and maintaining an independent research program, with emphasis on the immunological, inflammatory, or salivary aspects of the host response that will complement present investigators focused on the study of oral biofilms. Appropriate candidates with research interests in bioinformatics, functional genomics, proteomics, and computational biology will have the opportunity to work as a member of the New York State Center of Excellence in Bioinformatics and Life Sciences. Additional collaborative opportunities are available through the School of Medicine and Biomedical Sciences, Roswell Park Cancer Institute, and other affiliated organizations.

The successful candidate will be expected to contribute to the teaching mission of the Department, including supervision of graduate students in oral biology, and instruction in the undergraduate and graduate Dental School curricula. Successful candidates will hold D.D.S., D.M.D., M.D., Ph.D., or equivalent. Applications from individuals with dual degrees (e.g., D.D.S./Ph.D., D.M.D./Ph.D.) are especially encouraged. Candidates appointed will be expected to have significant grant funding, a national/international research reputation, and appropriate teaching experience. To apply, send your curriculum vitae, copies of three publications, a concise statement describing future research plans, and the names of three references to:

Dr. Kurt Winter
Department of Oral Biology
University at Buffalo
135 Foster Hall
Buffalo, NY 14214-3092

The University at Buffalo is committed to increasing diversity within its faculty by seeking women and minority candidates.

KEAN UNIVERSITY, UNION, NEW JERSEY
Department of Biological Sciences (Three Positions)

Tenure track positions effective September 1, 2006. Commitment to excellence in teaching required; ongoing agenda for research and publication expected. Specialization in:

Virology. To teach courses in virology and microbiology. Ph.D. required.

Ecology. To teach undergraduate courses in ecology, genes organisms and populations and course of specialty. Ph.D. required.

Botany. To teach principles of botany, elective courses in plant sciences; curate and maintain University Greenhouse. Ph.D. required.

Send letter of interest, resume, names and contact information of three references to:

Dr. Denise Mancarella, Chairperson
Dept of Biological Sciences
Kean University
1000 Morris Avenue
Union, NJ 07083

POSTDOCTORAL POSITION available immediately for **NEUROSCIENTIST** who is interested to study the functional mechanisms of communication between the basal forebrain and the cerebral cortex. postdoctoral will join a dynamic and creative neuroscience center at Rutgers-Newark **(website: http://www.cmbn.rutgers.edu)**, 13 miles from midtown Manhattan, New York City. Salary based on NIH postdoctoral scale. Preference will be given to candidates with experience, in patch clamping and imaging techniques. Send vitae, a description of research interests, and the names and contact information for three references to: **L. Zaborszky, MD, Ph.D. (e-mail: zaborszky@axon.rutgers.edu; website: http://zlab.rutgers.edu), Center for Molecular and Behavioral Neuroscience, Rutgers University, 197 University Avenue, Newark, NJ 07102.** *Rutgers University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSISTANT DEAN/DIRECTOR
The Richard Stockton College of New Jersey
Academic Laboratory and Field Facilities

The Division of Natural Sciences and Mathematics is seeking candidates for the position of Assistant Dean/Director for our Science Laboratories and Field Facilities.

Ph.D. in chemistry (or related area) plus management experience and a working knowledge of Occupational Safety and Health Administration, Chemical, Radiation, and Right-To-Know regulations required. The Director is responsible for the administration and management of laboratories and field facilities in all science disciplines; supervises eight full time and nine part-time professional employees; enforces and designs safety and operational procedures; functions as the College's Chemical Hygiene and Radiation Safety officer; monitors and maintains in conjunction with the Dean divisional budgets, initiates purchasing of equipment and supplies; coordinates facility renovations; and will have direct oversight in the construction of a new science center; is responsible for College-wide-chemical inventory database and implementation of Right-To-Know training and survey. Please refer to **website: http://www2.stockton.edu** for further information about the Division and its facilities as well as the position.

Screening will begin immediately with appointment for July 1, 2006. Send a letter of application, resume, and three letters of recommendation to: **Dean Dennis Weiss, Division of Natural Sciences and Mathematics, The Richard Stockton College of New Jersey, P.O. Box 195, Affirmative Action 29, Pomona, NJ 08240-0195.** *Stockton is an Affirmative Action/Equal Opportunity Employer.*

The Department of Psychiatry at the **University of Pennsylvania's School of Medicine** seeks candidates for an **ASSISTANT OR ASSOCIATE PROFESSOR** position in either the tenure-track or the non-tenure Clinician-Educator track. Track and rank will be commensurate with experience. The successful applicant will have experience in the field of neuropsychiatry with a focus on behavioral neuroscience, functional magnetic resonance imaging (fMRI). Applicants must have a Ph.D. or M.D. degree and have demonstrated excellent qualifications in education and research. Candidates should have research experience in application of fMRI or electrophysiology in human studies, preferably in area of memory and executive function. Individuals with training in developmental neuropsychology are encouraged to apply. Responsibilities include participation in multidisciplinary team of basic and clinical neuroscientists with opportunities to contribute to study of schizophrenia and other brain disorders while pursuing independent research. Opportunities for clinical and teaching activities are available. Please submit curriculum vitae and a letter of interest, along with three reference names to: **Dwight L. Evans, M.D., Professor and Chair; Raquel E. Gur, M.D., Ph.D.; REF #75, c/o A. Plotnick, Department of Psychiatry, University of Pennsylvania School of Medicine, 305 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021.**

The University of Pennsylvania is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.

SOIL MICROBIOLOGIST/CHEMIST. Oregon State University (OSU) seeks a full-time (1.00 full-time equivalent) Assistant or Associate Professor 12-month position (tenure-track offered at 0.75 full-time equivalent; 0.25 fixed-term funds) as a Soil Microbiologist or Soil Chemist to be part of a dynamic soil science community at OSU. For detailed position and application procedures, see **website: http://cropandsoil.oregonstate.edu/,** or contact: **Jayne Smith, Department of Crop and Soil Science, Oregon State University, 3017 ALS Building, Corvallis, OR 97331-7306. Telephone: 541-737-2441.** Application deadline is 1 April 2006. *OSU is an Affirmative Action/Equal Employment Opportunity Employer.*

POSITIONS OPEN

HOLDEN COMPREHENSIVE CANCER CENTER
The University of Iowa
Program Leader
Cancer Genetics and Computational Biology

The Holden Comprehensive Cancer Center (HCCC), an NCI-Designated Comprehensive Cancer Center at the University of Iowa, is seeking an established Scientist to lead the Cancer Center's Research Program in Cancer Genetics and Computational Biology. This program is a HCCC formal research program composed of 33 funded investigators from 17 departments. The diverse backgrounds of these investigators, coupled with their common focus on cancer genetics, provides a critical mass for productive scientific inquiry.

Resources available to the Program Leader include state-of-the-art laboratory space in the Roland and Ruby Holden Cancer Research Laboratories, which opened in late 2002, and membership in the University of Iowa Center for Bioinformatics and Computational Biology, a joint effort of the Carver College of Medicine and College of Engineering at the University of Iowa.

The successful candidate will have a track record of peer-reviewed research funding related to cancer genetics and/or computational biology, M.D. or Ph.D. degree, credentials for appointment at the Associate Professor or Professor level, and a demonstrated commitment to promoting a diverse environment. A primary academic appointment in a Department within the Carver College of Medicine at the University of Iowa is available and will be based on the expertise of the investigator.

Iowa City is located less than four hours by car from Chicago, St. Louis, Minneapolis, Omaha, and Kansas City and within 15 minutes of the Eastern Iowa airport. The city offers an array of cultural and athletic activities, including national theatre and symphony performances, international art exhibits, and Big Ten athletic events.

For more information, please visit our website at **website: http://www.uihealthcare.com/depts/cancercenter/.**

Applications should be sent to the following and should include curriculum vitae, statement of research interests and three letters of reference:

Genetics and Computational Biology
Search Committee
Holden Comprehensive Cancer Center
200 Hawkins Drive, 5970Z JPP
Iowa City, IA 52242-1002
E-mail: mary-jo-cooper@uiowa.edu
Telephone: 319-353-7898

Applicable background checks will be conducted.

The University of Iowa is an Equal Opportunity/Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

RESEARCH ASSISTANT PROFESSOR

Applications are invited for a research-track position that will involve 50 percent time dedicated to development of an individual's research career and 50 percent toward development of a Stem Cell/Sterile Cell Sorting/Flow Cytometry Core Facility in the University of South Carolina School of Medicine. The specific area of research should be in the areas of cancer, HIV/AIDS, or cardiovascular. The core facility will be a service facility developed to assist other principal investigators and their laboratories in experiments that involve these technologies. A strong background in immunology and a working knowledge of stem cell biology are essential. The review will begin immediately and continue until the position is filled. Applicants should submit their curriculum vitae, future research plans and three letters of reference to: **Dr. Robert Price, Director, Instrumentation Resource Facility, University of South Carolina School of Medicine, Columbia, SC 29208. E-mail: price@med.sc.edu.** *The University of South Carolina is an Affirmative Action, Equal Opportunity Institution.*

POSITIONS OPEN

FACULTY POSITION
Departments of Pharmacology and Psychiatry at Case School of Medicine/
University Hospitals of Cleveland
Cleveland, Ohio

The Department of Pharmacology (Case School of Medicine) in collaboration with the Department of Psychiatry (University Hospitals of Cleveland) seeks to identify a Faculty Member(s) to direct the development of a highly collaborative program of Neuropharmacology within the Department of Pharmacology. The program should address major problems of serious mental disorders, and complement ongoing clinical research within an NIMH-funded Bipolar Disorders Research Center in the Department of Psychiatry that focuses on the therapeutics and phenomenology of bipolar disorder, and includes child and adolescent psychiatry/psychology, adult psychiatry/psychology, addiction and geriatric psychiatry. The program should be integrated with the major growth of the Department of Pharmacology with the focus related to membrane biology. An externally funded, mid-career investigator will be appointed at the rank of Associate Professor or Full Professor in the tenure track and will receive an outstanding startup package commensurate with her/his "vision" for the program. Salary will be highly competitive and consistent with qualifications and current level of funding. Interested individuals should submit curriculum vitae, a brief statement of research interest(s), three representative reprints and the names, addresses, e-mail addresses and telephone numbers of three references electronically addressed to: **Ms. Cami Thompson** at **e-mail: cami@case.edu**

In employment, as in education, Case Western Reserve University and University Hospitals of Cleveland are committed to Equal Opportunity and World Class Diversity.

ASSISTANT PROFESSOR OF PHARMACOLOGY

The Department of Basic Pharmaceutical Sciences in the School of Pharmacy at the University of Louisiana at Monroe (ULM) invites applications for a twelve-month, tenure-track faculty position of Assistant Professor. This position includes an attractive recruitment package of salary, startup, and laboratory space. Candidates should have an earned doctorate in physiology/pharmacology and post-doctoral research experience, preferably in the areas of neuroscience, endocrinology, or cancer biology. The successful candidate is expected to develop an independent, externally funded research program, and contribute to teaching professional and graduate courses in the areas of physiology and pharmacology. Located in Monroe, a city whose metropolitan area population exceeds 100,000, the ULM campus offers a tranquil and cordial setting encompassing 238 acres, over 50 buildings, and an off-campus farm. Qualified individuals should submit their curriculum vitae, list of three references, and a statement of current interests and future goals emphasizing how their interests might complement the strengths of the Department to: **Karen P. Briski, Ph.D, Head, Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, 700 University Avenue, Monroe, LA 71209-0470, e-mail: briski@ulm.edu.** *The University of Louisiana at Monroe is an Equal Opportunity/Affirmative Action Employer.*

M.S./Ph.D. **PROTEIN BIOCHEMIST** with at least three years of experience in protein purification and analysis desired in the laboratory of **Dr. Richard Fishel, e-mail: rfishel@osu.edu.** Familiarity with routine PAGE/Western analysis, fast performance liquid chromatography purification, cellular overexpression, and at least a fundamental understanding of mass spectrometry, surface plasmon resonance, fluorescence anisotropy, and stop-flow/quench-flow kinetic studies. Salary will be based on experience and comparable to industry scales. *Only U.S. citizens and/or green card applicants will be considered.* To apply, please go to **website: http://www.jobsatosu.edu,** requisition 319514. *Equal Employment Opportunity/Affirmative Action Employer.*

Cold Spring Harbor Laboratory
2006 Meetings & Courses

Meetings

Neuronal Circuits: From Structure To Function
March 9 - 12 abstracts due: January 13
Edward Callaway, Dmitri Chklovskii, Liqun Luo

PTEN Pathways
March 15 - 19 abstracts due: January 20
Carlos Cordon-Cardo, Pier-Paolo Pandolfi, Ramon Parsons, William Sellers

Systems Biology: Global Regulation of Gene Expression
March 23 - 26 abstracts due: January 25
Peggy Farnham, Nir Friedman, Jack Keene

Channels, Receptors & Synapses
April 18 - 22 abstracts due: January 25
Richard Huganir, Lily Jan, Morgan Sheng

Gene Expression and Signalling in the Immune System
April 26 - 30 abstracts due: February 1
Doreen Cantrell, Richard Flavell, Rudolf Grosschedl, Stephen Smale

Molecular Chaperones & the Heat Shock Response
May 3 - 7 abstracts due: February 8
James Bardwell, David Ron, Jonathan Weissman

The Biology of Genomes
May 10 - 14 abstracts due: February 15
Kelly Frazer, Thomas Hudson, Svante Paabo, Richard Wilson

The Cell Cycle
May 17 - 21 abstracts due: February 22
Orna Cohen-Fix, Nicholas Dyson, David Morgan

Retroviruses
May 23 - 28 abstracts due: March 1
Frederic Bushman, Jaquelin Dudley

71st Symposium: Regulatory RNAs
May 31 - June 5 abstracts due: March 8
Bruce Stillman, David Stewart

Glia in Health & Disease
July 20 - 24 abstracts due: April 26
Ben Barres, Martin Raff

Mechanisms & Models of Cancer
August 16 - 20 abstracts due: May 24
Jacqueline Lees, Scott Lowe, Charles Sawyers, Charles Sherr

Molecular Genetics of Bacteria & Phages
August 22 - 27 abstracts due: May 31
Gary Dunny, Tina Henkin, Charles Turnbough, Jr.

Mouse Molecular Genetics
August 30 - September 3 abstracts due: June 7
Francois Guillemot, Janet Rossant, Hiroyuki Sasaki, Anthony Wynshaw-Boris

Translational Control
September 6 - 10 abstracts due: June 7
Alan Hinnebusch, Nahum Sonenberg, Gerhard Wagner

Axon Guidance, Synaptogenesis & Neural Plasticity
September 13 - 17 abstracts due: June 21
Anirvan Ghosh, Christine Holt, Mu-Ming Poo

Dynamic Organization of Nuclear Function
September 27 - October 1 abstracts due: July 5
Genevieve Almouzni, David Spector, Susan Wente

Molecular Genetics of Aging
October 4 - 8 abstracts due: July 12
Judith Campisi, Leonard Guarente, Gary Ruvkun

Germ Cells
October 11 - 15 abstracts due: July 19
Susan Strome, Azim Surani

Nuclear Receptors & Disease
November 2 - 5 abstracts due: August 18
Ronald Evans, Sohaib Khan, Keith Yamamoto

Pharmacogenomics
November 15 - 18 abstracts due: August 4
Alan Guttmacher, J. Steven Leeder, Debbie Nickerson, Munir Pirmohamed, Richard Weinshilboum, C. Roland Wolf

Neurodegenerative Diseases Biology & Therapeutics
November 30 - December 3
abstracts due: September 15
Sam Gandy, Virginia Lee, Marcy MacDonald, Peter Snyder

Spring Courses

applications due January 15

Protein Purification & Characterization
March 29 - April 11
Karen Adelman, Richard Burgess, Albert Courey, Sue-Hwa Lin

Cell & Developmental Biology of *Xenopus*
April 1 - 11
Janet Heasman, Christopher Wylie

Summer Courses

applications due: March 15

Genetics of Complex Human Diseases
June 7 - 13
Ammar Al-Chalabi, Laura Almasy

Advanced Bacterial Genetics
June 7 - 27
John Kirby, Susan Lovett, Anca Segall

Ion Channel Physiology
June 7 - 27
Mark Farrant, Michael Hausser, Nelson Spruston

Molecular Embryology of the Mouse
June 7 - 27
Blanche Capel, Michael Shen

Integrated Data Analysis for High Throughput Biology
June 14 - 27
Harmen Bussemaker, Vincent Carey, Partha Mitra, Mark Reimers, Anirvan Sengupta

Computational Neuroscience: Vision
June 16 - 29
Jonathan Demb, Eero Simoncelli, Stefan Treue

Proteomics
June 30 - July 13
Philip Andrews, Joshua La Baer, Andrew Link

Molecular Approaches to Plant Science
June 30 - July 20
Judith Bender, Lawrence Hobbie, Hong Ma, Sheila McCormick

Neurobiology of *Drosophila*
June 30 - July 20
Greg Bashaw, Scott Waddell, Bing Zhang

Mechanisms of Neural Differentiation & Brain Tumors
July 6 - 12
Abhijit Guha, Sadhan Majumder

Advanced Techniques in Molecular Neuroscience
July 6 - 20
James Eberwine, Thomas Hughes, Cary Lai

Biology of Social Cognition
(workshop)
July 14 - 20
Ralph Adolphs, David Skuse

Schizophrenia & Related Disorders
(workshop)
July 22 - August 1
David Lewis, David Porteous, Daniel Weinberger

Eukaryotic Gene Expression
July 25 - August 14
Michael Bulger, Thomas Oelgeschlager, Ali Shilatifard, Laszlo Tora

Imaging Structure & Function in the Nervous System
July 25 - August 14
Florian Engert, Mark Hubener, David Kleinfeld, Jack Waters

Yeast Genetics & Genomics
July 25 - August 14
Frank Luca, Jeffrey Strathern, Malcolm Whiteway

C. elegans
July 27 - August 14
Mario de Bono, Arshad Desai, Michel Labouesse

Stem Cells
August 3 - 16
Ronald McKay, Anne McLaren, Janet Rossant, Allan Spradling, Azim Surani, Max Wicha

Fall Courses

Applications due: June 15 * / July 15

X-Ray Methods in Structural Biology
October 16 - 31 *
William Furey, Gary Gilliland, Alexander McPherson, James Pflugrath

Programming for Biology
October 18 - 31
Suzanna Lewis, Simon Prochnik, Lincoln Stein, James Tisdall

Immunocytochemistry, In Situ Hybridization & Live Cell Imaging
October 23 - November 5 *
Abby Dernburg, John Murray, Jason Swedlow

Phage Display of Proteins & Peptides
November 7 - 20
Carlos Barbas, Don Siegel, Gregg Silverman

Computational & Comparative Genomics
November 8 - 14
William Pearson, Randall Smith

The Genome Access Course
April 25 - 26, August 29 - 30, November 29 - 30

above: a view of the CSHL beach from the Long Island Greenbelt Trail.

Cold Spring Harbor Laboratory
Meetings & Courses Program, 1 Bungtown Road, Cold Spring Harbor, NY 11724 phone 516 367 8346 fax 516 367 8845 email meetings@cshl.edu
http://meetings.cshl.edu

POSITIONS OPEN

CARDIOVASCULAR FACULTY POSITION

Applications are invited for a faculty position at the Cardiovascular Research Institute (CRI) directed **by Dr. A. Martin Gerdes** at the South Dakota Health Research Foundation (SDHRF), **website: http://www.sdhrf.org,** in Sioux Falls. This position will be filled at the **ASSISTANT, ASSOCIATE,** or **PROFESSOR** level. Currently, the CRI has seven faculty engaged primarily in studies of the molecular mechanisms of heart failure. Applicants with a background in vascular disease, diabetes, heart failure, or heart development are encouraged to apply. Applicants must have a Ph.D./M.D. or equivalent degree and a minimum of two years of postdoctoral experience. Competitive salaries, startup funds, and laboratory space will be provided. The new faculty member will have access to CRI Physiology, Imaging, Cell Culture, and Molecular Biology Cores. The SDHRF is a partnership between the Sanford School of Medicine of the University of South Dakota and Sioux Valley Hospital and Health Systems in Sioux Falls. Sioux Falls is a rapidly growing, affordable city of approximately 140,000 with excellent schools, low crime, low taxes, and excellent health care.

Application directions: Applicants should submit a letter of interest, curriculum vitae, and three letters of reference.

Application deadline: Applications will be accepted until the position is filled. Review of applications will begin on March 15, 2006. Submit to:

Betty Poppens, Ed.D., Director of Human Resources
South Dakota Health Research Foundation
1100 E. 21st Street, Suite 700
Sioux Falls, SD 57105
E-mail: bpoppens@usd.edu.

MARINE INVERTEBRATE PHYSIOLOGIST

Tenure-track **ASSISTANT PROFESSOR** to begin fall 2006; Ph.D. required. Applicants should be strongly committed to teaching and student-faculty research at the undergraduate level. Teaching responsibilities: marine invertebrate biology, comparative physiology, and additional courses in the marine science and biology majors. Participation in an interdisciplinary, values-oriented general education program is required, including a regular rotation in the two-semester freshman program. Send a letter discussing teaching and research interests and goals in working with undergraduates, along with curriculum vitae, teaching evaluations (if available), undergraduate and graduate transcripts, and three letters of recommendation to: **Dr. William A. Szelistowski, Galbraith Marine Science Laboratory, Eckerd College, 4200 54th Avenue S., St. Petersburg, FL 33711,** by March 15, 2006. **E-mail: szeliswa@eckerd.edu** (questions only). *Equal Opportunity Employer.*

DEPARTMENT OF BIOLOGY, DEPAUW UNIVERSITY, PHYSIOLOGIST. Applications invited for one-year term position beginning August 2006. Ph.D. preferred, all but dissertation considered. Teaching responsibilities include junior level animal physiology, introductory organismal biology, and introductory course in either ecology and evolution or cellular and molecular biology. Submit curriculum vitae, three letters of recommendation, transcripts, statement of teaching philosophy and interests, and evidence of teaching effectiveness to: **Physiology Search Committee, Department of Biology, DePauw University, Greencastle, IN 46135.** Review of applications will begin March 1, 2006, and continue until position is filled. *DePauw University is an Affirmative Action, Equal Opportunity Employer. Women and members of under-represented groups are encouraged to apply.*

ASSISTANT PROFESSOR
Evolutionary Genetics
Queens College of the City University of New York

The Department of Biology at Queens College of the City University of New York seeks a tenure-track Assistant Professor to begin September 1, 2006. We seek candidates with a doctoral degree, postdoctoral experience, and a record of research accomplishment in the area of evolutionary genetics. Suitable research areas include population genetics, comparative genomics, and molecular ecology, and related fields. Successful candidates will be expected to establish an externally funded research program and teach at the undergraduate and graduate (M.A./Ph.D.) levels. Please submit a cover letter, curriculum vitae, a two-to-three-page research plan, a statement of teaching interest, and arrange for submission of three current letters of recommendation. Candidates must submit materials by March 27, 2006, to: **Dr. Stephane Boissinot, Chair, Evolutionary Genetics Search Committee, Department of Biology, Queens College of CUNY, 65-30 Kissena Boulevard, Flushing, NY 11367-1597.** *An Equal Opportunity/Affirmative Action/IRCA/Americans with Disabilities Act Employer.*

SERVICES

WANT TO AVOID VISA BACKLOGS IN APPLYING FOR PERMANENT RESIDENCY? If you have a job offer and are internationally recognized in your field, you may be eligible to obtain a green card based on your exceptional ability without waiting for an available visa. Contact **David M. Kramer, Esq.,** certified specialist and selected Super Lawyer in Immigration Law by his peers, for a free consultation. Visit the law offices of **David M. Kramer** at **website: http://www.kramervisalaw.com,** or **telephone: 818-907-9688; fax: 818-386-2335; e-mail: dmk@ix.netcom.com.**

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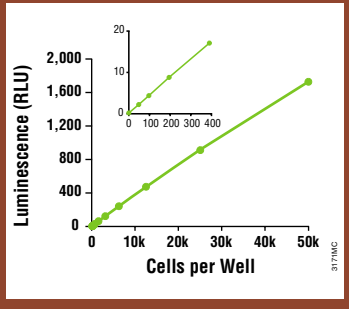

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